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Diaminopimelic Acid Analogues as Inhibitors of Enzymes Involved In Bacterial Lysine Biosynthesis

by

Jennifer Fay Caplan

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

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Department of Chemistry

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You gain strength, courage and confidence by every experience
in which you really stop and look fear in the face…
You must do the thing which you think you cannot do.

-Eleanor Roosevelt

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Diaminopimelic Acid Analogues as Inhibitors of Enzymes Involved In Bacterial Lysine Biosynthesis by Jennifer Fay Caplan in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The synthesis of five *meso*-2,6-diaminopimelic acid (*meso*-DAP) analogues from glycine derivatives and their testing against key bacterial enzymes involved in L-lysine biosynthesis is described.

Target 5-((2*S*)-amino-2-carboxy-ethyl)-isoxazole-3-carboxylic acid (**31**) is prepared using a 1,3-dipolar cycloaddition reaction of ethyl chlorooximidoacetate (**46**) with methyl (2*S*)-2-(*N*-(benzyloxycarbonyl)amino)-4-pentenoate (**34**) followed by oxidation with active manganese dioxide as the key transformations. An improved synthesis for the isoxazole moiety can be realized *via* cycloaddition of **46** with methyl (2*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-4-pentynoate (**79**), providing methyl (2S)-2-((*N*-*tert*-butoxycarbonyl)amino)-3-(3-(ethoxycarbonyl)isoxazole)-2-yl-propanoate (**80**) in one step. Reductive ring opening of **80** with molybdenum hexacarbonyl, followed by amino and diester deprotection gives cyclic vinylogous amide (6*S*)-4-oxo-1,2,3,4-tetrahydropyridine-2,6-dicarboxylate (**84**) and open chain vinylogous amide (6*S*)-2,6-diamino-4-oxo-2-heptene-1,7-dicarboxylate (**85**).

Isoxazole DAP analogue **31** and vinylogous amides **84** and **85** show weak inhibition against *meso*-DAP dehydrogenase and DAP epimerase suggesting that a flexible substrate-analogue may be required in order to effectively inhibit these enzymes. Cyclic vinylogous amide **84**, structurally similar to L-THDP **7**, is a strong competitive inhibitor (K_i 32 μM) of L-DHDP reductase. Since vinylogous amide **84** possesses key structural elements of betanidin **201,** studies toward the total synthesis of this natural product were undertaken.

(2*RS*,6*S*)-2,6-Diaminoheptane-1,7-dioic acid, 7-(*N*-hydroxy)amide (**94ab**) is synthesized by ene reaction of **34** with methyl glyoxylate (**96**), followed by Mitsunobu reaction with *tert*-butyl *N*-((2-(trimethylsilyl)ethyl)sulfonyl)carbamate (**103**). Deprotection of the amide, subsequent reaction with hydroxylamine, followed by removal of the remaining protecting groups affords the desired hydroxamate (12% overall yield). The *N,N´*-dimethylamino DAP derivative **173ab** can also be prepared from **34** in an analogous manner. *N*-Succinyl-DAP hydroxamate **130ab** is synthesized from 2,2,2-trichloroethyl (2*S*)-2-(*N*-(benzyloxycarbonyl)amino)-4-pentenoate (**147**) in 10 steps (7% overall yield) using ene and Mitsunobu chemistry.

Enzyme studies reveal that hydroxamate **130ab** is not an inhibitor of the zinc-dependent DAP desuccinylase and that *N,N´*-dimethyl **173ab** displays no inhibition against *meso*-DAP dehydrogenase, DAP epimerase or DAP decarboxylase. Hydroxamic acid **94ab** is a substrate for both *meso*-DAP dehydrogenase (K_m = 1.39 mM ± 0.17 mM, V_{max} = 0.026 mM/min ± 0.002 mM/min) and DAP epimerase. Further, hydroxamate **94ab** shows 82% inhibition at 5 mM against *meso*-DAP adding enzyme and is the only analogue from this work to inhibit DAP decarboxylase (IC_{50} 0.35 mM) suggesting that decarboxylase has highly specific substrate requirements.

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LIST OF ABBREVIATIONS

[α]	specific rotation
abs	absorbance units
Anal.	analysis
APT	attached proton test
aq	aqueous
AT	aminotransferase
atm	atmosphere
AZIDAP	2-(4-amino-4-carboxybutyl)-2-aziridine-carboxylate
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
br	broad
tert-Bu	*tert*-butyl
n-BuLi	*n*-butyl lithium
calcd	calculated
Cbz	benyloxycarbonyl
Cbz-Cl	benzyl choroformate
CI	chemical ionization
Conc.	concentrated
COSY	correlated spectroscopy

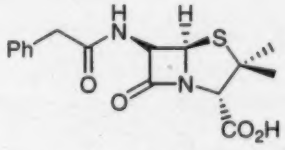
δ	chemical shift in parts per million downfield from TMS
d	doublet
DAP	2,6-diaminopimelic acid
DBU	1,4-diazobicyclo[2.2.2]octane
DCC	1,3-dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DEAE	diethylaminoethyl
DH	dehydrogenase
DMAP	4-*N,N*-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DTT	dithiothreitol
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EDTA	ethylenediaminetetraacetic acid
EI	electron impact ionization
ESI	electrospray ionization
EPIM	epimerase
Et_2O	diethyl ether
FAB	fast atom bombardment
GlcNAc	*N*-acetylglucosamine
HPLC	high performance liquid chromatography
IC_{50}	concentration causing 50% inhibition
IR	infrared
J	coupling constant

k_{cat}	catalytic rate constant
K_i	inhibition constant
K_m	Michaelis-Menten constant
LDA	lithium diisopropyl amide
LHMDS	lithium hexamethyldisilazane
m	multiplet
MeCN	acetonitrile
MEOH	methanol
m/z	mass to charge ratio
MHz	megahertz
min	minute(s)
mp	melting point
MS	mass spectrometry
MurNAc	*N*-acetylmuramic acid
MW	molecular weight
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
nm	nanometers
NOE	nuclear overhouser effect
Nu	nucleophile
PLP	pyridoxal phosphate
ppm	parts per million
Py	pyridine

q	quartet
qui	quintet
R_f	retardation factor
RP	reverse phase
rt	room temperature
s	singlet
Ses	2-((trimethylsilyl)ethyl)sulfonyl
t	triplet
TBAF	tertabutylammonium fluoride
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THDP	tetrahydrodipicolinate
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N,N,N´,N´*-tetramethylethylenediamine
TMS	trimethylsilane
TMSCl	trimethylsilyl chloride
TMSI	trimethylsilyl iodide
Tris-HCl	tris-(hydroxymethyl)aminomethane hydrochloride
p-TsOH	*p*-toluenesulfonic acid
U	unit
UV	ultraviolet
V	velocity
V_{max}	maximal enzyme velocity

INTRODUCTION

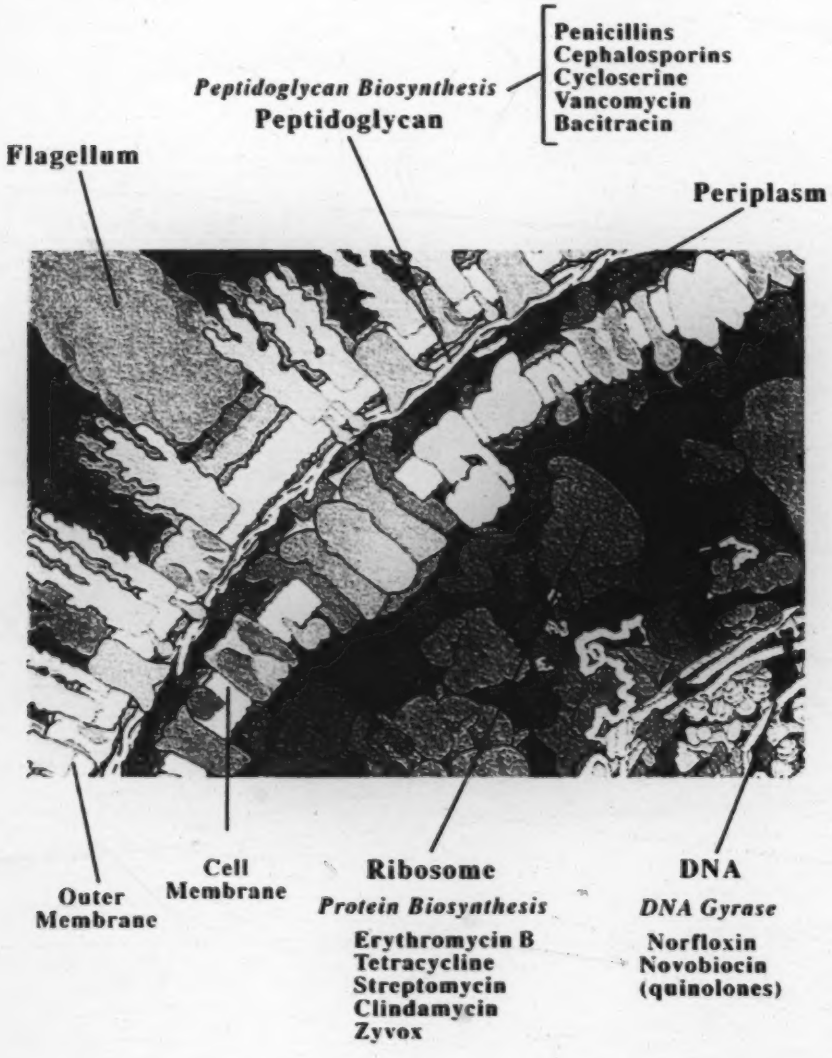
The scientific battle waged against antibiotic resistance in the treatment of infectious diseases remains one of the greatest challenges of the 21st century. The beginning of the antibiotic era in the 1930's saw the use of sulfonamides against pneumonia and the introduction of penicillin **1**[1] during World War II. These antibiotics were monumental to man's triumph over bacteria and marked a new era of medicine against disease.



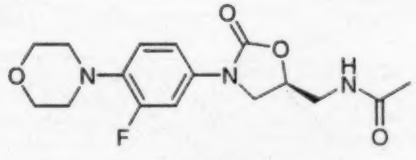
Penicillin **1**

Antimicrobial chemotherapies during the past sixty years included a wide variety of drugs aimed at specific physiological targets in the bacterial cell (Figure 1). Recently however, an alarming increase in antimicrobial resistance has emerged as a serious health problem,[2,3] threatening the clinical efficacy of the most widely used antibiotics, including the β-lactams and glycopeptides.[4,5] One of the most fierce bacterial enemies we face today is *Staphylococcus aureus*, a species that has managed to evade not only penicillin, but also other classical antibiotics such as tetracycline and erythromycin B.[6] Furthermore, a number of top-selling antibiotics worldwide are in danger of becoming obsolete by the advancement of antibiotic resistance.[7]

Figure 1. Diagram of a cross-section of an *E. coli* bacterial cell magnified one million times[a] showing the physiological targets of several clinically used antibiotics.



Antibiotic resistance is prevalent due to a number of factors. Firstly, microbes possess resistance genes. Each resistance gene expresses a protein which nullifies the damaging effect of the antimicrobial agent on its target site in the bacterial cell. Subsequent gene transfer to other organisms then results in rapid proliferation of resistance within the bacterial kingdom.[9,10] Secondly, many antibiotics are prepared by semi-synthetic methods, being derived from bacteria and fungi, wherein resistance mechanisms operate to protect the producing organism. Modification of known antibiotics has been the major source of new medicinal agents for decades. Thus resistance mechanisms often work against an entire class of compounds. Although completely novel classes of antibiotics were discovered and frequently implemented into clinical practice throughout the 1940's and 1950's, a truly new antibiotic class did not emerge for many years until the recent introduction of Zyvox (Linezolid) **2**, a member of the oxazolidinone class.[11,12,13]



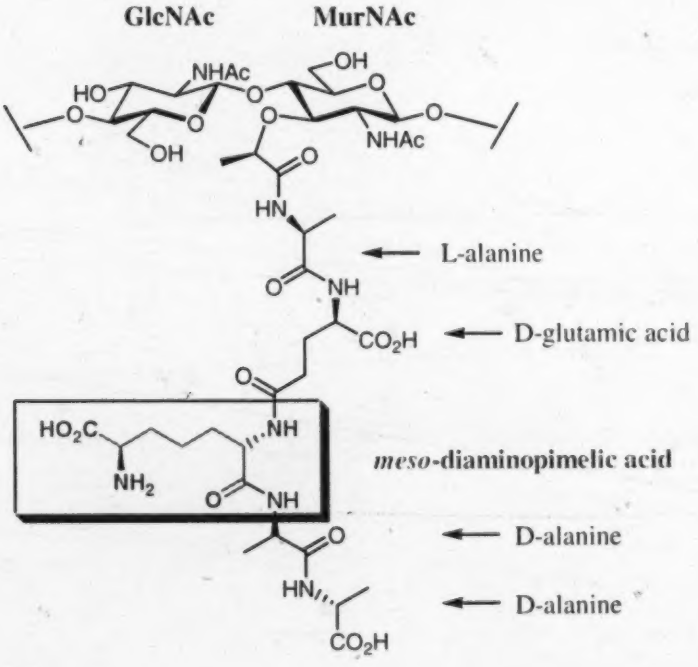
Zyvox **2**

The above factors highlight the need for an alternative design of new classes of antimicrobial agents rather than the development of more drugs based on existing antibiotics. Since the inhibition of peptidoglycan biosynthesis has long been an attractive target for antibacterial design,[14,15,16] many new potential antimicrobials are targetted toward this physiological process.

1. Peptidoglycan

Peptidoglycan, or murein, is a complex matrix of carbohydrate chains that surrounds the bacterial cell membrane and is comprised of alternating β-1,4-linked *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) sugar residues, cross-linked *via* pentapeptide sidechains appended to the muramyl moiety (Figure 2).[17]

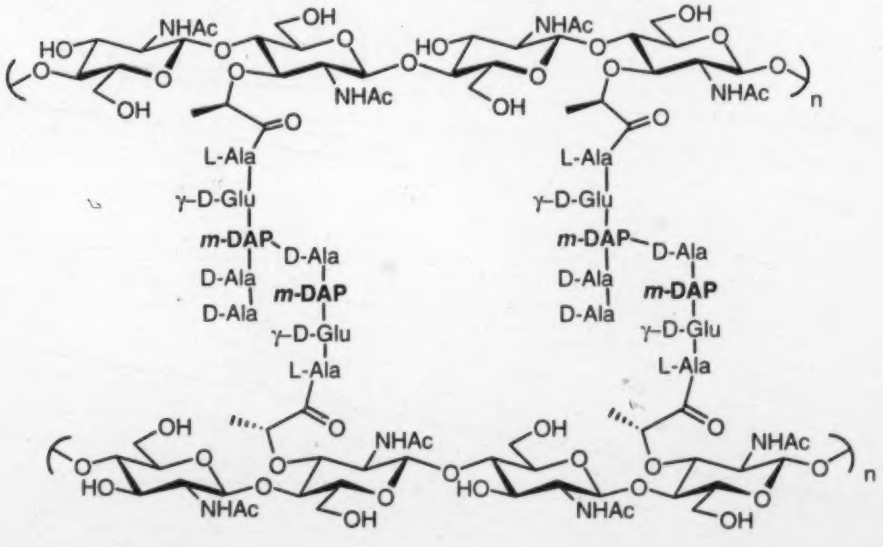
Figure 2. Peptidoglycan monomer in Gram-negative bacteria.



Murein provides strength and rigidity, protecting the cells from lysing under high internal osmotic pressures. The internal Turgor pressure has been estimated to be 5 atm for Gram-negative bacteria and as much as 30 atm in Gram-positive microbes.[18] The pentapeptide side chain contains D-amino acids that stabilize peptidoglycan against enzymatic hydrolysis. The general structure of the pentapeptide is L-Ala-γ-D-Glu-**X**-D-Ala-D-Ala, where **X** is an L-amino acid containing an amino group in the side chain. Crucially, Gram-negative bacteria require **X** to be *meso*-diaminopimelic acid[19,20] (*meso*-DAP) whereas its biosynthetic product, L-lysine, has an analogous function in many Gram-positive organisms. Some variations in the identity of this cross-linking moiety do exist however in numerous bacterial species.[20,21]

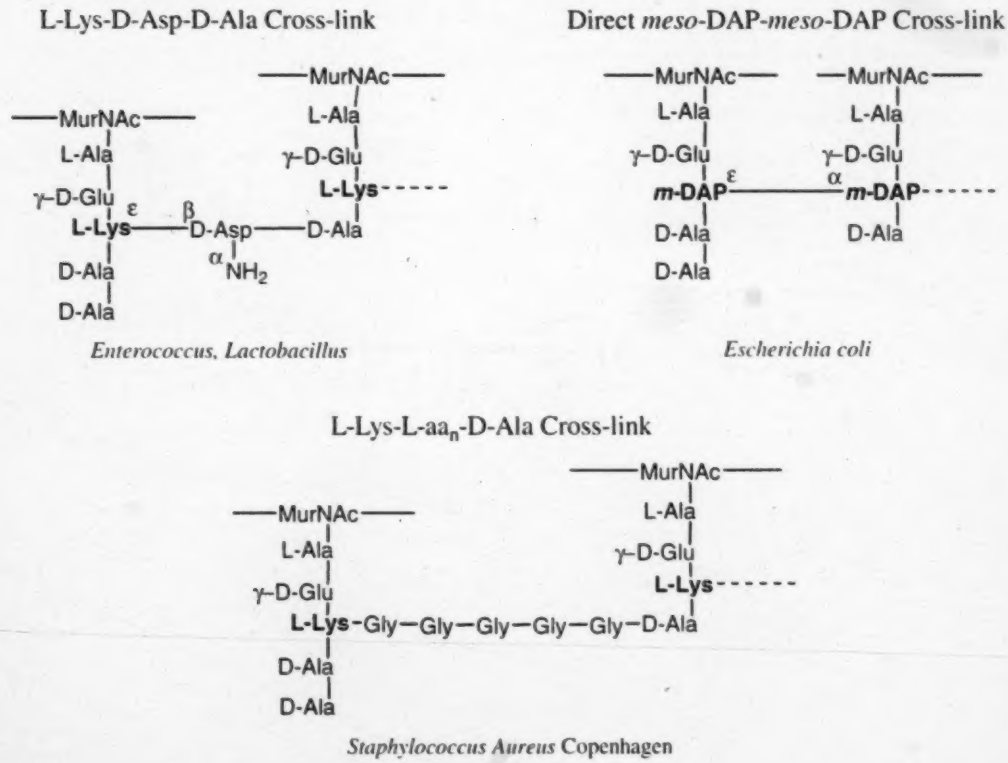
The peptidoglycan strands are held together by a network of peptide cross-links formed between adjacent pentapeptide chains (Figure 3). The resilience and structural

Figure 3. Peptidoglycan layer in Gram-negative bacteria.



rigidity of the murein is due to the level of cross-linking. In Gram-negative bacteria such as *E. coli*, the degree of cross-linking is in the range of 25-50%, whereas in the multi-layered cell wall of Gram-positive bacteria, the level of cross-linking is 70-90%.[22] The simplest type of these 'inter-bridges' involve direct formation of a peptide bond between the penultimate D-alanine of one peptide with the free ω-amino group of the diamino acid, either L-lysine or *meso*-DAP, in an adjacent glycan chain. The composition of this peptide linkage varies greatly between bacteria[21] with some representative examples illustrated in Figure 4.[16]

Figure 4. Peptide cross-links through *meso*-DAP or L-lysine at position 3.

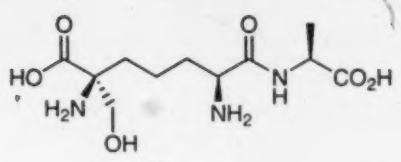


Many of the best antibiotics today function by inhibiting peptidoglycan biosynthesis (Table 1). β-Lactams,[23] such as the cephalosporins and penicillins, D-cycloserine,[24] bacitracin and vancomycin[25] all have powerful antibiotic properties. Because resistance to these and other antibiotics is increasing rapidly,[2-5] there has been a resurgence of interest in studying the enzymes involved in peptidoglycan biosynthesis. As demonstrated in Figures 2-4, the diamino acid residue located at the third position of the pentapeptide chain in peptidoglycan, often DAP or lysine, plays a pivotal role in the integrity of the murein sacculus as it is directly involved in the peptide cross-linkages.

Table 1. Peptidoglycan biosynthesis as a target for antibiotics.

Antibiotic	Target
β-Lactams	Transpeptidation of peptidoglycan
D-Cycloserine	Alanine racemase/ D-Ala-D-Ala ligase
Bacitracin	Translocation across membrane
Vancomycin	Binding of peptidyl-D-Ala-D-Ala

The biosynthesis of L-lysine *via* diaminopimelic acid appears not to be a target for naturally occurring antibiotics.[26,27,28] However, an α-hydroxymethyl DAP analogue isolated from *Micromonospora chalcea*, alanyl dipeptide **3**, shows antibiotic activity against *E. coli*.[29]



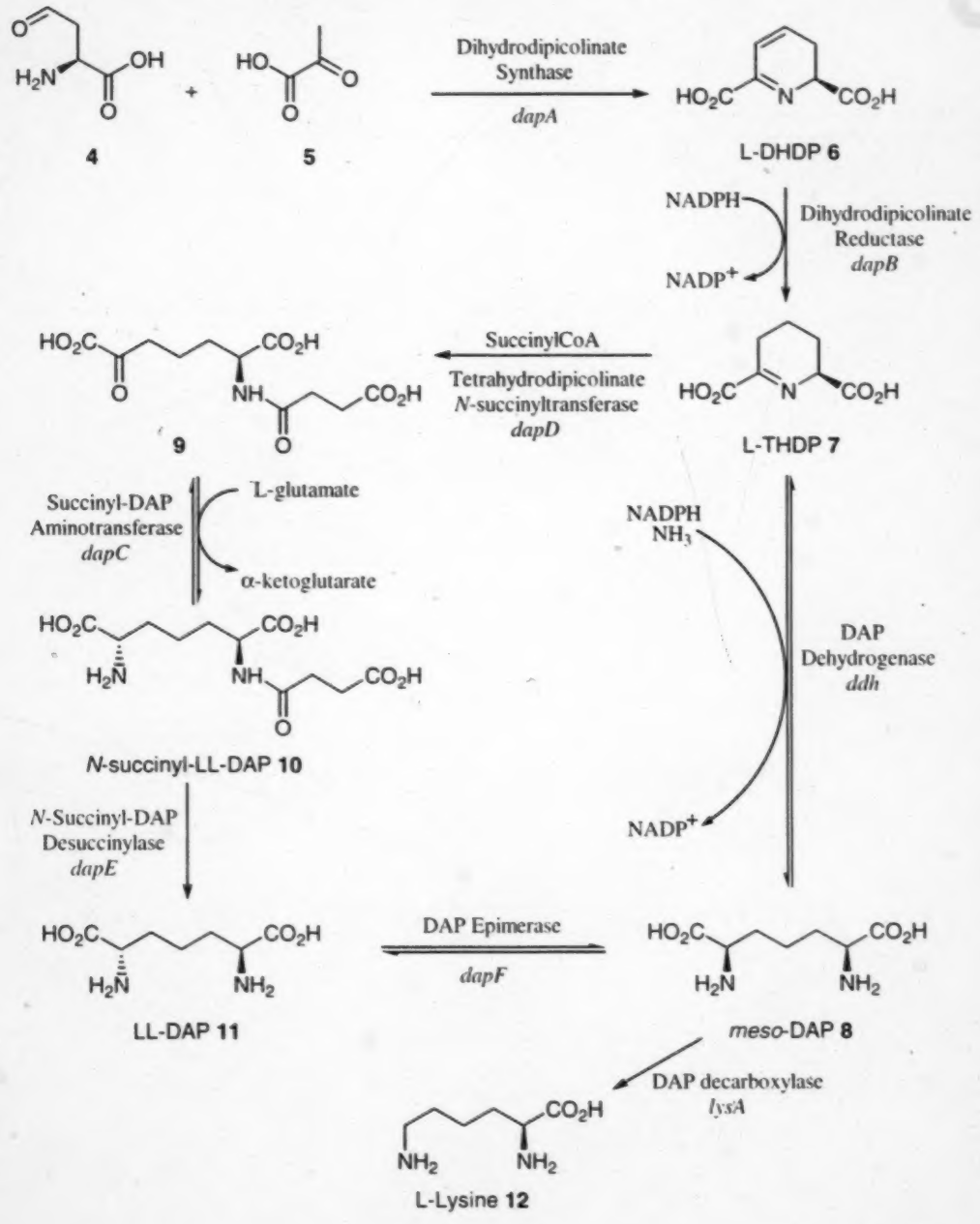
3

The absence of the peptidoglycan network in mammals, who require a dietary source of L-lysine, implies that the design of inhibitors of the DAP biosynthetic pathway should provide drugs with selective toxicity against bacteria. The search for new broad spectrum antimicrobial agents has thus encouraged our group and others to undertake extensive studies on enzymes and inhibitors of the diaminopimelate (DAP) pathway to lysine.[21-30] It is hoped that structural and mechanistic information on these essential bacterial enzymes will facilitate the rational design of new antibiotics.

2. Bacterial Lysine Biosynthesis

The biosynthesis of L-lysine in bacteria proceeds *via* a series of nine enzyme-catalyzed reactions (Scheme 1).[31] The first step of the pathway involves the condensation of L-aspartate-semialdehyde (**4**) with pyruvate **5**, resulting in the formation of L-1,2-dihydrodipicolinate (L-DHDP) (**6**). Reduction of this intermediate by the L-DHDP reductase leads to the formation of L-tetrahydrodipicolinate (L-THDP) (**7**). At this point the biosynthetic pathway diverges in three different directions, ultimately leading to the formation of DAP and lysine.[32]

Scheme 1. L-Lysine biosynthetic pathway in prokaryotes.



The less common dehydrogenase pathway involves the direct conversion of L-THDP **7** to *meso*-DAP **8** *via* an NADPH-dependent reductive amination catalyzed by DAP dehydrogenase. This pathway has been discovered in only a few strains of bacteria such as *Corynebacterium glutamicum*, *Bacillus sphaericus* and a *Pseudomonas* and *Brevibacterium* species.[32,33,34,35] The succinylase pathway, used by all Gram negative and many Gram positive bacteria for the production of lysine,[35,36] involves the use of *N*-succinylated intermediates to produce *meso*-DAP **8**. In this route, as outlined in Scheme 1, the nitrogen of L-THDP **7** acquires a succinyl moiety from succinyl-CoA to produce an acyl-blocked α-amino-ε-ketopimelate (**9**). In some cases, *N*-acetylated intermediates are used instead but this appears to be limited to the *Bacillus* species, with the acetylase pathway of *B. megaterium* being the most well characterized system.[33,34] Ketopimelate **9** then undergoes transamination by the pyridoxal phosphate (PLP)-dependent DAP aminotransferase, using glutamate as the amino donor, to provide a second amino center of L-configuration.[37] Desuccinylation of intermediate **10** *via* the action of DAP desuccinylase furnishes LL-DAP **11** which is then epimerised using *meso*-DAP epimerase to give the lysine precursor, *meso*-DAP **8**. The final step of the pathway involves PLP-dependent *meso*-DAP decarboxylase which catalyses the decarboxylation of *meso*-DAP **8** to give L-lysine (**12**). In several bacterial species more than one biosynthetic pathway operates. For example the industrially important lysine producer, *Corynebacterim glutamicum*, utilizes both the succinyl-blocked and the dehydrogenase pathways.[38,39] Thus, the presence of multiple biosynthetic pathways suggests the importance of DAP and lysine to bacterial survival.

Recently, the cloning of the biosynthetic genes and overexpression of many of the enzymes involved in bacterial lysine biosynthesis has facilitated detailed studies, including extensive kinetic investigations as well as the determination of enzyme structures *via* X-ray crystallography.[27-40] Several enzymes in the DAP pathway either utilize substrates having an imine bond or have transient intermediates wherein the α-carbon center in the amino acid moiety becomes planar. Among these are the dihydrodipicolinate (DHDP) reductase, DAP D-dehydrogenase, and DAP epimerase.[26,41]

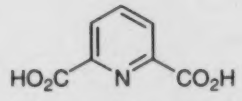
Two other DAP enzymes of interest include the PLP-dependent DAP decarboxylase and the zinc-dependent DAP desuccinylase. Rational design of potent inhibitors for these bacterial enzymes requires a more detailed knowledge of enzyme mechanism and structure.

3. Key Bacterial Enzymes Involved in Lysine Biosynthesis

3.1 Dihydrodipicolinate Reductase (EC 1.3.1.26)

In DAP biosynthesis, the pyridine-nucleotide dependent enzyme dihydrodipicolinate reductase (DHPR), encoded by the *dapB* gene, reduces the α,β-unsaturated cyclic imine α-dihydrodipicolinate L-DHDP **6** to L-THDP **7** (Scheme 1). Farkas and Gilvarg initially identified the enzyme in *E. coli*.[42] To date, the gene encoding the reductase has been sequenced from a wide variety of bacteria including Gram-negative, Gram-positive and mycobacterial species.[43] The most studied reductase thus far is the *E. coli* enzyme. The L-DHDP reductase enzyme from *E. coli* is a homotetramer

possessing a molecular weight (M_r) of 115, 032 daltons. Overexpression of the protein in *E. coli* has allowed detailed mechanistic and crystallographic studies.[44] The X-ray crystal structure of the enzyme subunit obtained at 2.2 Å resolution reveals that the enzyme possesses both a co-factor and a substrate binding domain. On the basis of a crystal structure of a binary enzyme–NADPH complex[45] and deuterium exchange kinetics,[46] the amino terminal domain has been shown to bind the dinucleotide whereas the carboxyl terminus is proposed to bind the substrate. The crystal structure suggests that the domains may move to adopt either an 'open' or 'closed' conformation, rendering NADPH closer to the bound substrate as needed for the catalysis. The recently reported three-dimensional structure of a ternary *E. coli* enzyme–NADH–2,6-pyridinedicarboxylate complex confirms the involvement of the carboxyl terminus in substrate binding.[47] The DHDP analogue 2,6-pyridinedicarboxylate **13** is the best known inhibitor of the enzyme to date with a competitive inhibition constant (K_i) of 26 μM.



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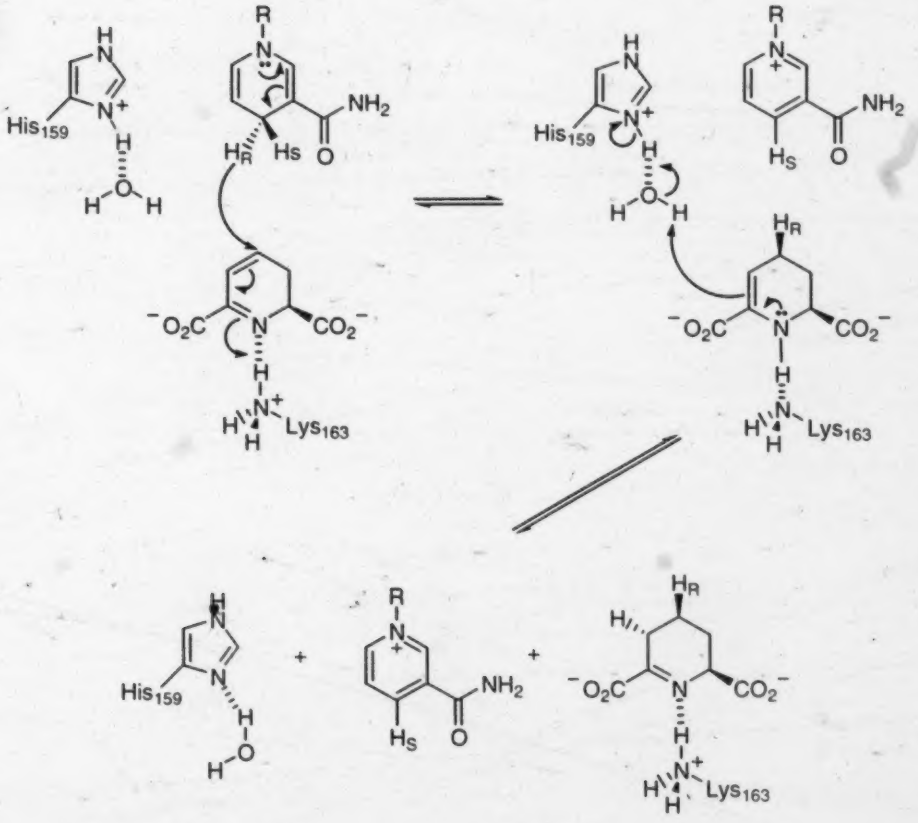
Michaelis constants have been determined for both the substrate L-THDP **7** (K_m = 50 μM) and the co-factor NADPH (K_m = 8 μM). Based on the kinetic and structural information, a mechanism has been proposed for the DHDP reductase enzyme (Scheme 2).[47] The stereochemistry of the hydride transfer was investigated using both [4*S*]- and [4*R*-4-^{3}H]-NADPH and subsequently determining the transferred radioactivity in

chromatographically separated products. These results show that the *E. coli* DHDP reductase catalyzes the transfer of the *pro-R* hydrogen to the substrate.

Scheme 2. Proposed mechanism for DHDP reductase.



3.2 *meso*-Diaminopimelate Dehydrogenase (EC 1.4.1.16)

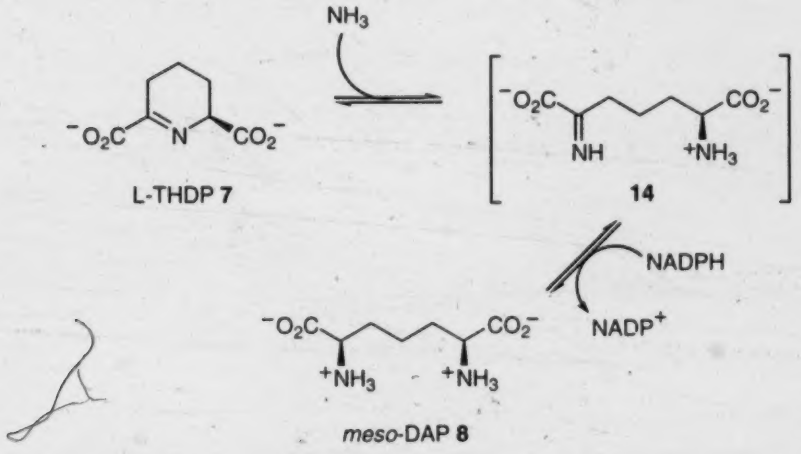
Meso-diaminopimelate dehydrogenase catalyzes the direct conversion of L-THDP **7** into *meso*-DAP **8** (Scheme 1). DAP dehydrogenase belongs to a large family of pyridine dinucleotide-dependent enzymes that catalyze the reductive amination of an α-keto acid to its corresponding amino acid.[48] However, DAP dehydrogenase is unique among the amino acid dehydrogenases as it is the only enzyme which stereospecifically reduces an imine to form a D stereocenter.

The enzyme is specific for the *meso* isomer of DAP, and neither D,D- nor L,L-DAP are substrates for the enzyme. Remarkably, for the reaction to proceed, the enzyme must be able to distinguish between two stereochemically opposite centers on the same symmetrical molecule of *meso*-DAP **8**. The first report of DAP dehydrogenase activity was discovered in *Bacillus sphaericus*.[49] Detailed kinetic analysis of the *Bacillus* enzyme has revealed that the reaction is sequentially ordered.[50] Like the classical glutamate dehydrogenase,[51] the forward reaction involves NADPH binding first followed by L-THDP **7** and then ammonia. Upon completion of the reaction, *meso*-DAP **8** is released first, followed by $NADP^+$.

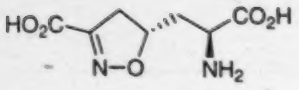
The forward reaction is believed to proceed *via* ring opening of L-THDP **7** by ammonia, generating planar imine intermediate **14** (Scheme 3) which is reduced stereospecifically by the 4-*pro*-*S* hydrogen of NADPH to furnish the D-stereocenter of *meso*-DAP **8**. Rational design of potent enzyme inhibitors is often dictated by the structure of the natural substrate or the structures of the transition states formed during

the course of an enzyme-catalyzed reaction. Inhibitors mimicking the bound imine intermediate **14** would thus be expected to bind tightly to the dehydrogenase.

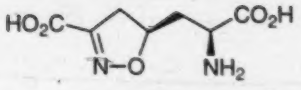
Scheme 3. Proposed mechanism for *meso*-DAP dehydrogenase.



The synthesis of such inhibitors has been reported and, in particular, the (2*S*,5*S*)-isoxazoline **15** was shown to be a potent reversible inhibitor of the *B. spaericus* DAP dehydrogenase and to possess antibacterial activity.[52] The inhibition constant (K_i) for the reductive amination was 4.2 μM, while the corresponding value for the oxidative deamination reaction was 23 μM at pH 7.8. In contrast, the diastereomer (2*S*,5*R*)-isoxazoline **16** fails to display any significant inhibition of the dehydrogenase reaction at millimolar concentrations.



15

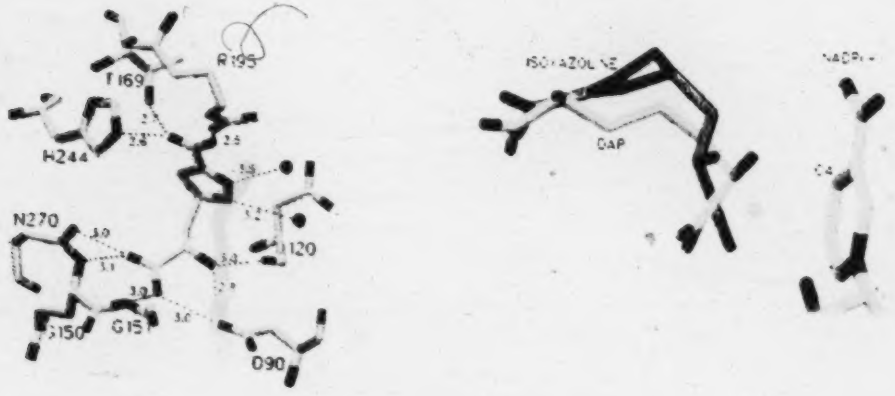


16

The *ddh* gene encoding the *Corynebacterium glutamicum* DAP dehydrogenase has been sequenced,[53] expressed in high levels in *E. coli*[54] and the three-dimensional crystal structure of the binary enzyme–$NADP^+$ complex solved to 2.2 Å resolution.[55] The enzyme has three main domains, one binding the substrate, one binding NADPH and the last one forming the 'dimerization' domain between the two monomers of the enzyme. Furthermore, hydrogen/deuterium exchange experiments suggest that certain domains of the protein 'close' upon substrate binding, rendering the substrate and co-factor appropriately oriented for reduction to occur.[56] Additional information has been gained from the X-ray crystal structure of isoxazoline inhibitor **15** bound *meso*-DAP dehydrogenase.[57] The DAP binding site was fully revealed by this binary complex, showing that the compound bound in an *all-trans* conformation and explaining the unique selectivity for the *meso*-DAP isomer. Interestingly, the isoxazoline **15** binds in an unexpected fashion in the active site of the enzyme with the isoxazoline ring bound in the distal L-binding pocket, thus orienting the L-amino acid end of the molecule towards the nicotinamide ring of the co-factor where the reaction occurs (Figure 5). The α-proton of the L-center is rendered away from $NADP^+$ and thus the reaction cannot proceed.

Figure 5. (a) Isoxazoline **15** bound-DAP dehydrogenase (b) Overlay of DAP and **15** in DAP dehydrogenase substrate binding site.

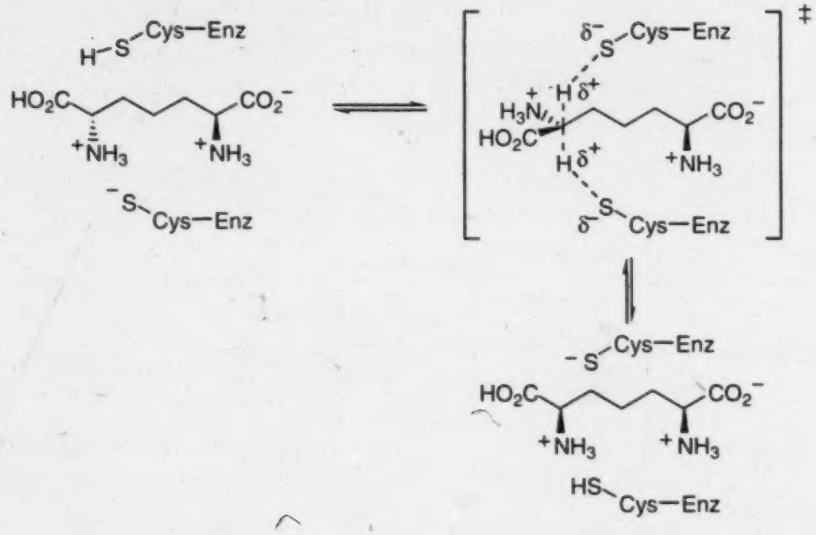
(a) (b)



3.3 Diaminopimelate Epimerase (EC 5.1.1.7)

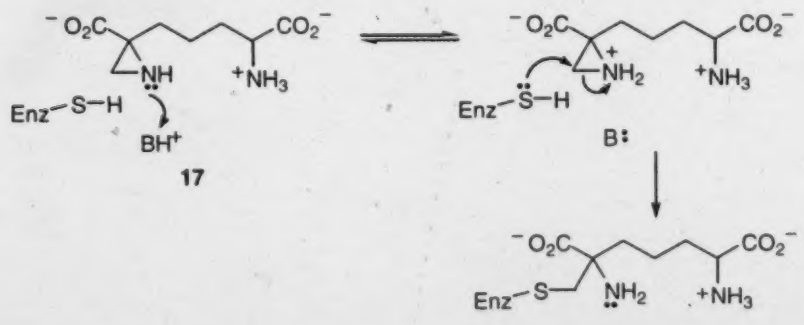
Diaminopimelate epimerase, a member of the non-pyridoxal phosphate-dependent amino acid racemases, catalyzes the interconversion of L,L and D,L-*meso*-diaminopimelic acid.[58] Like the well-studied PLP-independent amino acid racemases, proline racemase,[59] glutamate racemase[60] and aspartate racemase,[61] DAP epimerase does not require the aid of co-factors or metal ions but rather uses two cysteine residues for catalytic activity. The thiolate of one cysteine residue acts as a general base, abstracting the α-proton from one face while the second thiolate acts as a general acid and delivers the proton to the opposite face, resulting in inversion of stereochemistry at the α-position (Scheme 4).

Scheme 4. Proposed mechanism for DAP epimerase.



An early mechanistic study of the *E. coli* diaminopimelate epimerase supported a 'two-base' mechanism.[58] Further evidence which suggested that a cysteine is present at or near the active site was the irreversible inactivation of the *E. coli* enzyme at the Cys73 position by 2-(4-amino-4-carboxybutyl)-aziridine-2-carboxylate (azi-DAP) (**17**) (Scheme 5).[62]

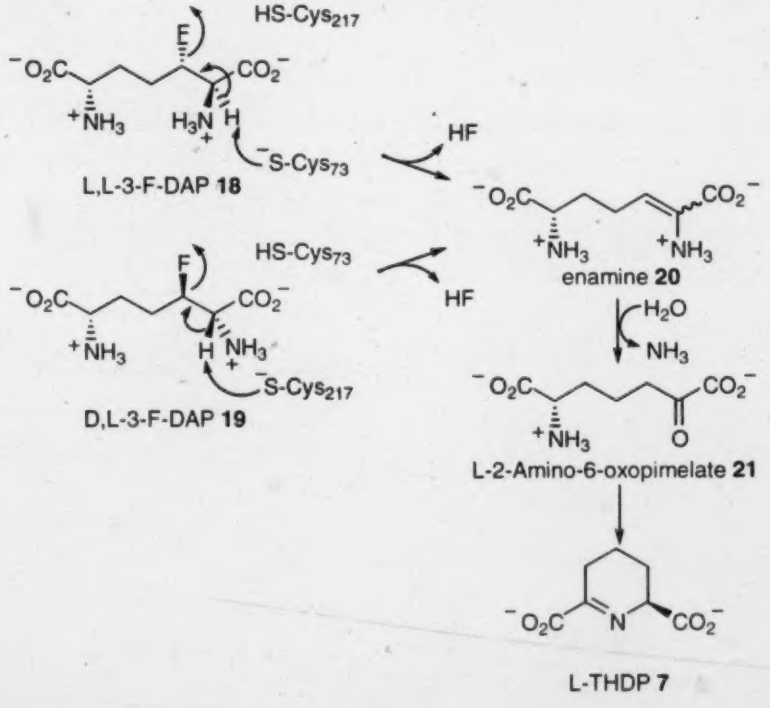
Scheme 5. Irreversible inactivation of DAP epimerase by azi-DAP **17**.



The three-dimensional crystal structure of the *Haemophilus influenzae dapF*-encoded[62a,63,64] diaminopimelate epimerase reveals a monomeric (M_r 30, 265), two-domain enzyme of 274 amino acids.[65] The oxidized inactive enzyme has two-conserved cysteine residues, Cys73 and Cys217 in a disulfide linkage at the interface of the two structurally superimposable domains. Kinetic and isotopic studies of the reduced active enzyme suggest that these two cysteine residues act as the catalytic acid and base.[66]

More recently, on the basis of investigations using cysteine site-directed mutants of *H. influenzae* DAP epimerase along with diastereomeric 3-fluoro-DAP substrates **18** and **19**, Cys73 and Cys217 have been assigned as the general base for proton abstraction in the L,L → D,L and the D,L → L,L directions, respectively (Scheme 6).[67]

Scheme 6. Proposed mechanism of hydrogen fluoride elimination by DAP epimerase.



3.4 Diaminopimelate Desuccinylase (EC 3.5.1.18)

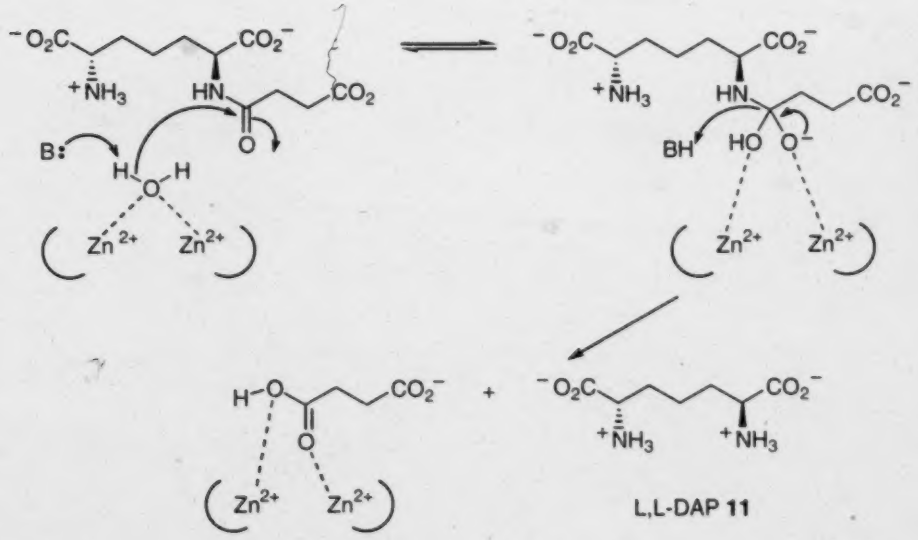
The *dapE*-encoded desuccinylase catalyzes the hydrolysis of *N*-succinyl-L,L-diaminopimelic acid (**10**), forming L,L-diaminopimelic acid (**11**) and succinate. Previous studies on the *E. coli* enzyme suggested that the desuccinylase exists as a homodimer and that the addition of either zinc or cobalt increases the enzyme activity.[68,69] These early studies also indicated that DAP desuccinylase was intolerant to changes in substrate structure. In particular, the enzyme was specific for an L-amino center at the distal end of *N*-succinyl-L,L-diaminopimelic acid (**10**) and did not accept *N*-acetyl-L,L-DAP as a substrate.

The amino acid sequences of bacterial desuccinylases have been reported for *E. coli*,[70] *H. influenzae*,[71] *C. glutamicum*,[72] *Helicobacter pylori*,[73] and *Mycobacterium tuberculosis*.[74] Recently, the *dapE* gene was cloned from *H. influenzae*, overexpressed to high levels in *E. coli*, purified to homogeneity, and detailed kinetic investigations were performed.[75] An alignment of the known *dapE* sequences identified a number of residues that are highly conserved in a variety of metal-dependent enzymes.[76] The well-characterized carboxypeptidase G2,[77] whose three-dimensional crystal structure reveals the presence of two zinc atoms at the active site, shows strong sequence homology with the *dapE*-encoded desuccinylase. It was therefore proposed that the active site of DAP desuccinylase contains two metal ions, one of which is tightly bound. The high affinity site is usually occupied by zinc, while a second atom of zinc or cobalt can occupy the low affinity site. Also, metal stoichiometries determined by inductively-coupled plasma mass spectrometry (ICP-MS) studies suggest that a single metal atom from the enzyme is able

to catalyze the desuccinylation reaction, however the maximal velocity is achieved upon metal binding to both sites.[75] Steady-state kinetic experiments have shown that the enzyme has a turnover number of 200 s^{-1} with a K_m for *N*-succinyl-L,L-DAP of 1.3 mM.

A mechanism which is similar to those proposed for other metal-dependent amidases has been proposed for the DAP desuccinylase (Scheme 7).[75] In the proposed mechanism, water activated by zinc acts as the nucleophile generating a tetrahedral intermediate that is transiently stabilized, then collapses with protonation of the departing amine.

Scheme 7. Proposed mechanism for the *dapE*-encoded desuccinylase.



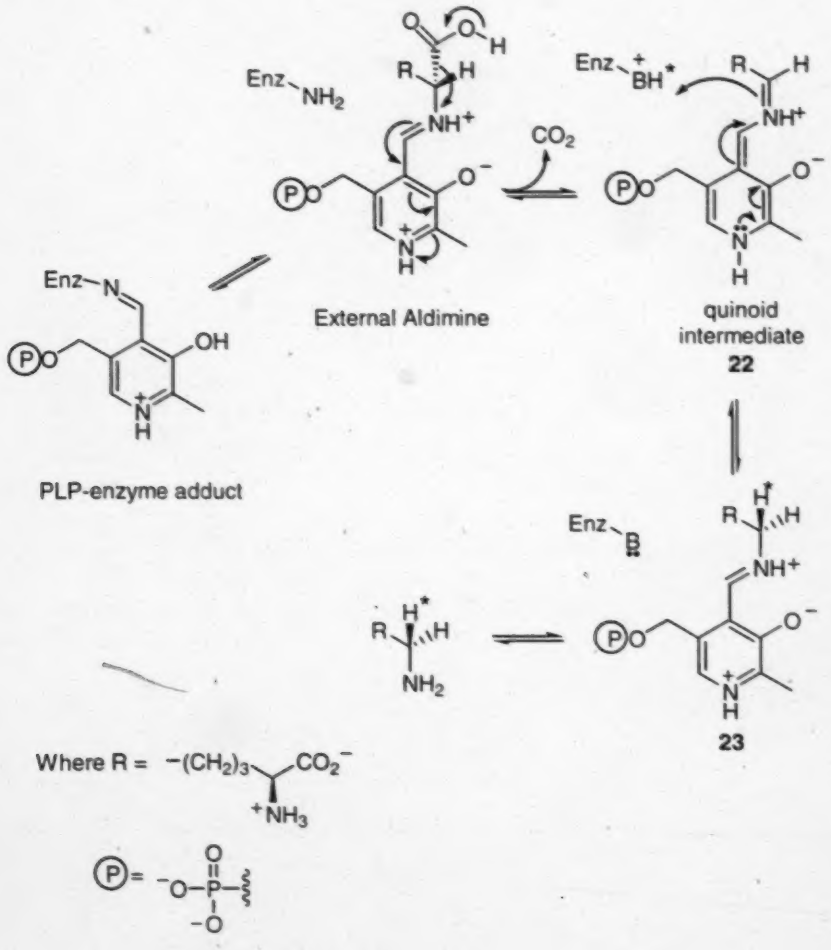
3.5 Diaminopimelate Decarboxylase (EC 4.1.1.20)

Meso-diaminopimelate decarboxylase is a pyridoxal 5'-phosphate (PLP) dependent enzyme, isolated from both plants[78,79,80] and bacteria,[81,82,83] that catalyzes decarboxylation at the D-center of *meso*-DAP **8**. The K_m for *meso*-DAP is approximately 1.7 mM for most bacterial decarboxylases. Over the past decade, numerous bacterial *lysA* genes have appeared in the literature, including those from *E. coli*,[84] *C. glutamicum*,[85] *B. subtilis*,[86] and *P. aeruginosa*.[87] Alignment of these bacterial decarboxylase sequences shows three conserved lysine residues, one of which is believed to be the lysine that binds the PLP cofactor in an aldimine linkage.[41] Investigation of DAP decarboxylase from *B. sphaericus*[88] and wheat germ[89] revealed that, unlike other PLP dependent decarboxylases[90,91,92] where the reaction is accompanied by retention of stereochemistry, this reaction occurs with an unusual inversion of configuration to give L-lysine (**12**). The overall geometrical outcome could be accounted for by a mechanism similar to other PLP decarboxylases, which involves the formation of a common quinoid intermediate **22** in the enzyme active site (Scheme 8).

Decarboxylation proceeds from an external aldimine complex in which the carboxyl group is oriented such that the carboxyl-α-carbon bond is perpendicular to the plane of the conjugated π-system. The carbanion generated after decarboxylation is resonance stabilized by electron delocalization into the pyridinium ring nitrogen, forming quinoid intermediate **22**. Protonation of this quinoid tautomer from the *re* face at the

α-carbon proceeds with overall stereochemical inversion, leading to the formation of the L-lysine-PLP imine **23** which is transiminated prior to product release.

Scheme 8. Reaction catalyzed by PLP dependent *meso*-DAP decarboxylase.



4. Project Goals: Design of DAP Enzyme Inhibitors

Previous mechanistic, kinetic and crystallographic studies of the enzymes involved in bacterial lysine biosynthesis provide valuable tools to aid in the design and synthesis of inhibitors that mimic transient intermediates or transition state analogues. Since enzymes of the DAP pathway tend to have high substrate specificity and excellent stereochemical recognition at the distal (non-reacting) site, design of potent inhibitors is a challenging task that requires incorporation of numerous functionalities into a small molecule.[30]

The elucidation of the mechanisms of L-DHDP reductase, DAP epimerase and DAP dehydrogenase suggests that DAP analogues possessing an sp^2-hybridized planar α-carbon at the reactive site may be inhibitors of these enzymes. Further, the proposed mechanism for DAP decarboxylase implies the design of inhibitors that may interfere with the formation of the external aldimine or competitively bind in the enzyme active site without undergoing a decarboxylation reaction. The mechanism for the metal-catalyzed hydrolysis by DAP desuccinylase suggests the design of inhibitors which may interfere with metal binding in the enzyme active site.

The objective of this work is to examine the interaction of several novel diaminopimelate analogues with key bacterial enzymes of the DAP pathway utilizing both synthetic organic chemistry and protein biochemistry tools. All the synthetic analogues will possess an L-distal configuration that is required for enzyme recognition.

This research will focus on the investigation of the following enzymes: DAP epimerase, DAP dehydrogenase, DHDP reductase, DAP desuccinylase, and DAP decarboxylase.

It is hoped that the investigations presented in this thesis will provide new information concerning the mechanisms of these bacterial enzymes and lead to novel and more potent inhibitors that may be potential antimicrobial agents.

RESULTS AND DISCUSSION

1. Olefinic DAP Analogues as Enzyme Inhibitors

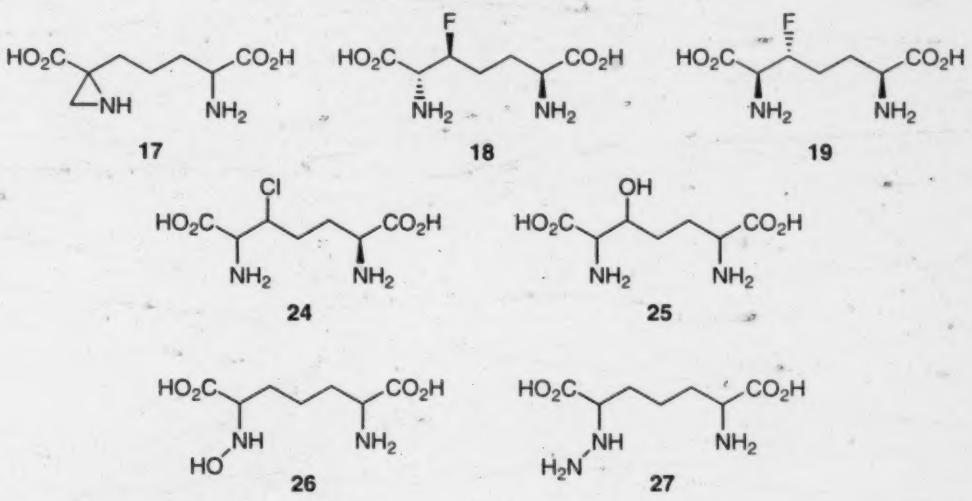
Over the past two decades, numerous DAP derivatives have been synthesized and studied for inhibition activity against key bacterial enzymes involved in lysine metabolism.[30]

Meso-DAP epimerase and dehydrogenase are believed to function by related mechanisms where planar character develops at the α-carbon of the DAP skeleton in the transition state of each reaction (Scheme 3 and 4). As a consequence, amino acid derivatives possessing the same reactive moieties could inactivate these enzymes. DAP analogues are therefore designed to mimic these putative planar transition states, as in the case of DAP epimerase, or contain the appropriate leaving groups, such as a β-carbon or *N*-amino substituent, to generate enzyme-catalyzed elimination products (Figure 6).

The β-fluoro-DAP diastereomers **18** and **19** are potent inhibitors of the epimerase enzyme, having IC_{50} values of 4 and 25 μM respectively.[93] The HF elimination catalyzed by the epimerase generates enamine **20** that is planar at the reacting α-carbon and thus a mimic of the postulated transition state. A diastereomeric mixture of β-chloro-DAP **24** inhibits the epimerase with a K_i value of 200 nM.[94] Like the β-fluoro-DAP analogues **18** and **19**, the elimination of the HCl is catalyzed by the epimerase to produce intermediate **20** which cyclizes to give L-THDP **7** (Scheme 9). In contrast, the β-hydroxy-DAP

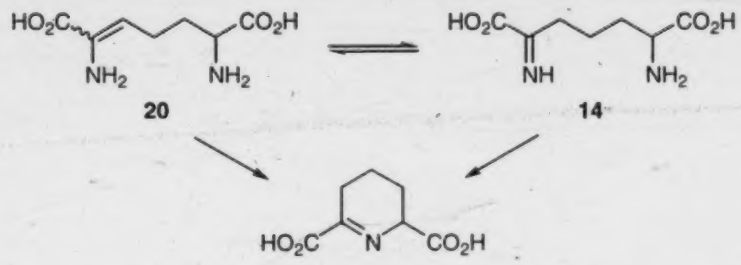
isomers **25**, which do not undergo enzyme-catalyzed elimination of water, exhibit only weak inhibition of epimerase with IC_{50} values in the millimolar range.[93,94,95]

Figure 6. DAP analogues synthesized to inhibit DAP epimerase and dehydrogenase.



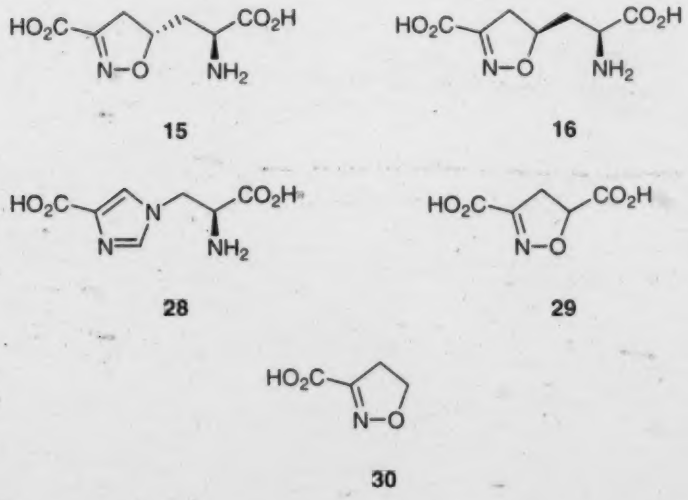
A diastereomeric mixture of *N*-hydroxy-DAP **26** reversibly and competitively inhibits the epimerase enzyme with a K_i value of 5.6 μM.[95] The analogous *N*-amino-DAP **27** however is a poor inhibitor of the enzyme (K_i 2.9 mM). It is believed that the enzyme catalyzed elimination of water from **26** leads to the formation of imine **14**, the proposed intermediate in the conversion of *meso*-DAP **8** to L-THDP **7** by *meso*-DAP dehydrogenase. As depicted in Scheme 9, both enamine **20** and imine **14** can cyclize to give L-THDP **7**. However, only one of the compounds shown in Figure 6, mimic **26**, is an inhibitor of the dehydrogenase enzyme (K_i 84 μM).

Scheme 9. Cylization of enamine **20** and imine **14** to L-THDP.



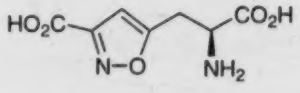
Heterocyclic derivatives of DAP possessing a planar α-carbon center at the reactive end of the molecule have also been investigated as potential inhibitors of DAP epimerase and *meso*-DAP dehydrogenase (Figure 7).[52]

Figure 7. Heterocyclic DAP derivatives.



Of the heterocycles depicted, isoxazoline **15** is the only compound that displays potent inhibition of *meso*-DAP dehydrogenase (K_i 23 μM). The amino acid side chain and the stereochemistry of its attachment are critical for inhibition, as evidenced by the fact that neither diastereomer **16** nor derivatives **28-30** display significant inhibition of the dehydrogenase reaction at millimolar concentrations. In addition, the compounds shown in Figure 7 are only weak inhibitors of the DAP epimerase enzyme ($IC_{50} > 1$ mM).

To further investigate the interaction of heterocyclic DAP analogues with *meso*-DAP dehydrogenase and epimerase, the synthesis of isoxazole **31** was proposed. This aromatic DAP derivative may provide additional information with regards to both stereochemical requirements and importance of particular amino acid moieties in binding to *meso*-DAP dehydrogenase.

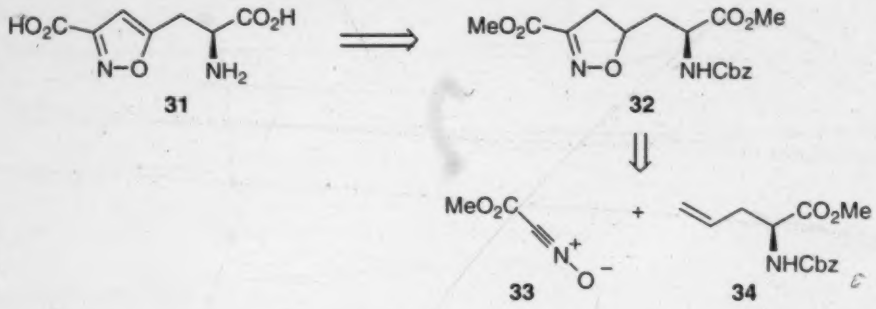


31

1.1 Synthesis of Isoxazole DAP Analogue 31

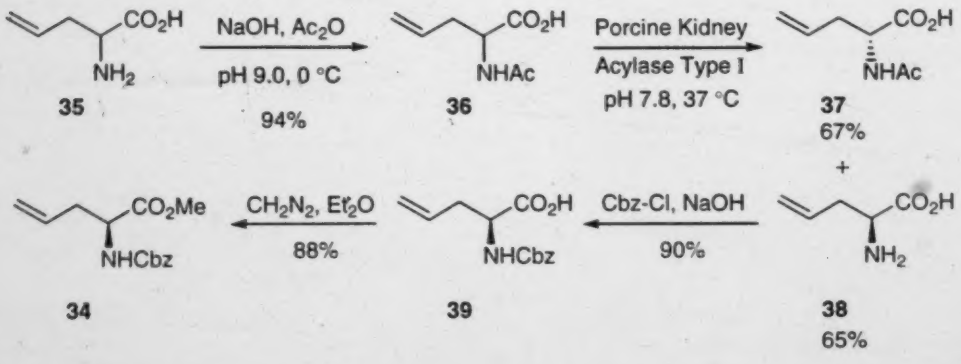
From a retrosynthetic perspective, isoxazole **31** may be formed *via* oxidation of the corresponding isoxazoline **32** (Scheme 10). The construction of this isoxazoline moiety involves a cycloaddition reaction between nitrile oxide **33** and allylglycinate **34**.[52]

Scheme 10. Retrosynthetic scheme for synthesis of isoxazole **31**.



The required glycinate **34** can be synthesized as outlined in Scheme 11.[52] Acylation of racemic D,L-allylglycine (**35**) with acetic anhydride gives derivative **36** in 94% yield. Enzymatic resolution of racemic D,L-*N*-acetyl- allylglycine (**36**) is then achieved using Porcine Kidney Acylase Type I which selectively hydrolyzes the *N*-acetyl amide bond of L-amino acids.[97] Cation exchange chromatography results in the separation of the two compounds to give D-*N*-acetylallylglycine (**37**) and L-allylglycine (**38**) in 67% and 65% yields respectively (Scheme 11).

Scheme 11. Synthesis of allylglycinate **34**.

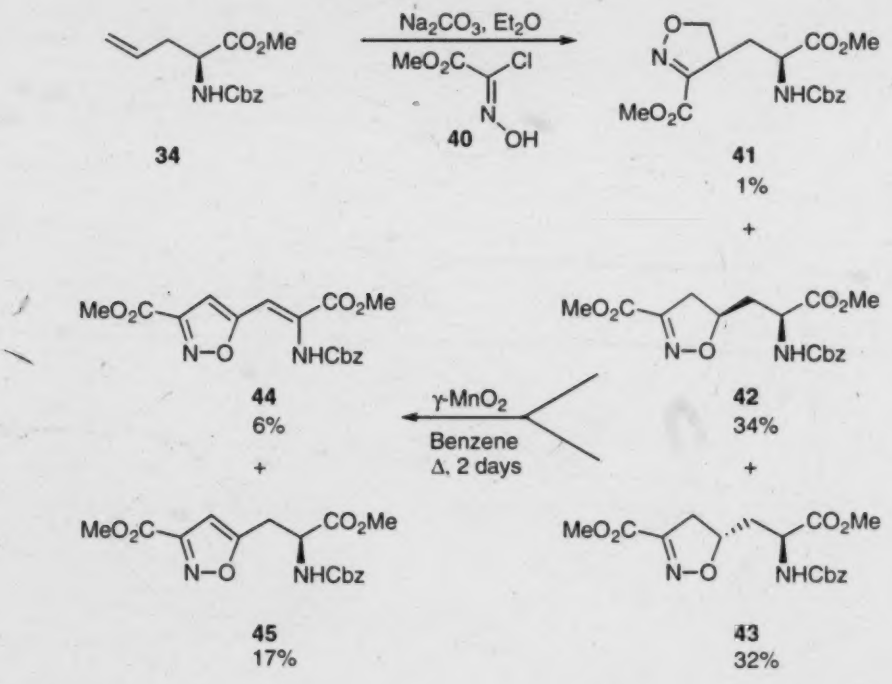


Protection of L-allylglycine as its *N*-Cbz methyl ester derivative **34** is achieved according to literature procedures,[98] providing **34** in 50% overall yield from **35**. Subsequent reaction of **32** with nitrile oxide **33** generated *in situ* by base treatment with methyl chlorooximidoacetate (**40**) synthesized using a known procedure,[99] generates the framework of the isoxazoline analogue (Scheme 12). This known cycloaddition reaction gives two major diastereoisomers **42** (34%) and **43** (32%) in addition to a small amount of regioisomer **41** (1%) whose stereochemistry is not determined.[52]

With the isoxazoline moiety in place, oxidation is required to furnish the isoxazole ring. Several reagents have been reported in the literature to effect this transformation including the use of DDQ[100,101,102] and freshly precipitated manganese dioxide.[103,104,105] A variety of methods for preparing manganese dioxide have been described resulting in variable activity.[106,107,108] Well-defined types of manganese dioxide, in particular, that precipitated from manganese sulfate in the presence of alkali as described by Attenburrow[109] and active γ-manganese dioxide prepared as described by Fatiadi[106] were thus chosen for attempted synthesis of compound **31**.

Isoxazolines **42** or **43** are transformed to the desired isoxazole **45** in 17% yield using a ten-fold excess, by weight, of activated manganese dioxide prepared by either of the above methods in refluxing benzene containing activated molecular sieves. Recovered starting material (50%) and side-product **44** (6%) are also obtained.

Scheme 12. 1,3-Dipolar cycloaddition reaction of **34** with nitrile oxide **40**.

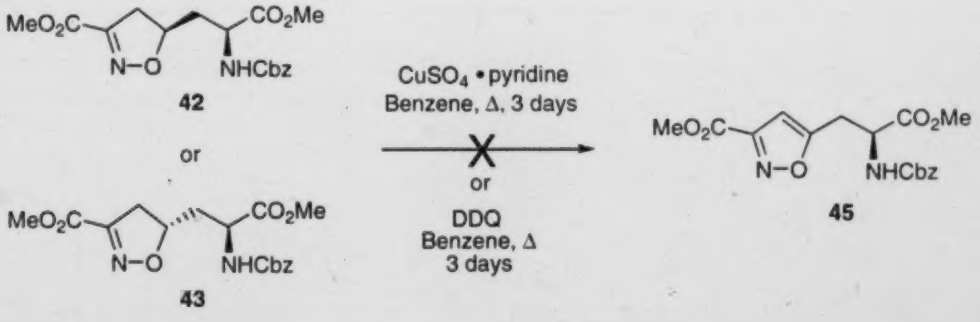


Previous studies indicate that a large excess of the oxidant and prolonged reaction times are required to achieve complete transformation and that azeotropic removal of the water formed in the reaction by means of suitable solvents is critical to product yield. The reaction is believed to proceed through a series of electron transfers to manganese, with the initial step being the adsorption of substrate.[106] In an effort to optimize this oxidation, a variety of reaction conditions were investigated. It has been suggested that the correct choice of solvent is essential in this reaction because many solvents can compete with the substrate for coordination sites present on the surface of active manganese dioxide.

A reaction using either activated form of manganese dioxide employing a variety of solvents such as chloroform, toluene, petroleum ether and ethyl acetate does not alter the yield. Furthermore, azeotropic removal of the water formed using a Dean-Stark trap in reactions containing toluene or benzene has no significant effect on the efficiency of the oxidation. Many isoxazolines successfully transformed to isoxazoles in the literature possess a phenyl or double bond functionality as a substituent at position 5 in the ring. The poor yields obtained may thus be attributed to the lack of this conjugation in isoxazolines **42** and **43**.

In light of these results, an attempt was made to synthesize the desired isoxazole by treating isoxazoline **43** with DDQ in benzene and refluxing for 3 days according to the procedure of Das *et al.*,[108] as illustrated in Scheme 13. The reaction was monitored by TLC and unfortunately failed to give the desired product. Reaction of **43** with copper sulfate/ pyridine in refluxing benzene also gave none of the expected isoxazole **45**.

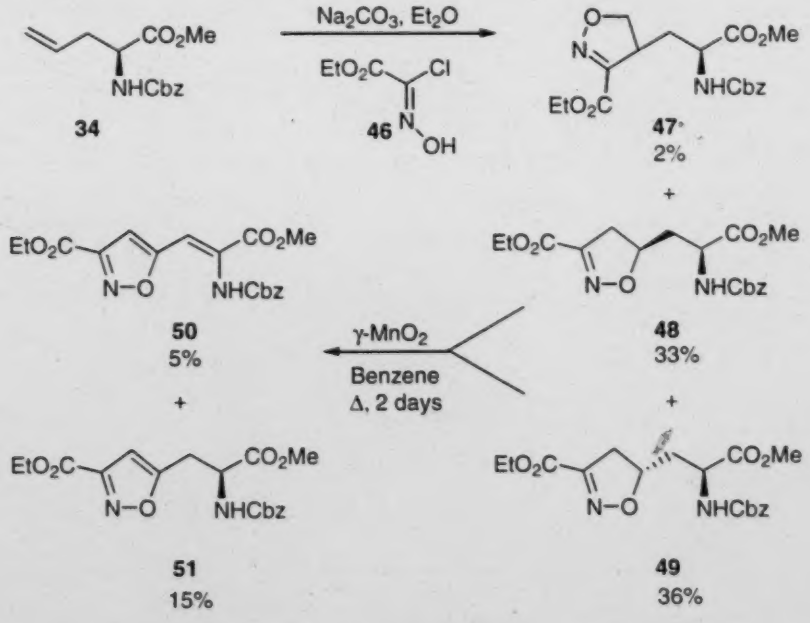
Scheme 13. Attempted oxidation of **42** and **43** with copper sulfate or DDQ.



In order to produce substantial quantities of isoxazole **45** for completion of the synthesis, the manganese dioxide procedure would require a large amount of starting isoxazolines **42** and **43**. The synthesis and recrystallization of methyl chlorooximidoacetate (**40**)[110] used in the isoxazoline preparation can be problematic. Hence the ethyl chlorooximidoacetate (**46**) was synthesized and used for further investigations.

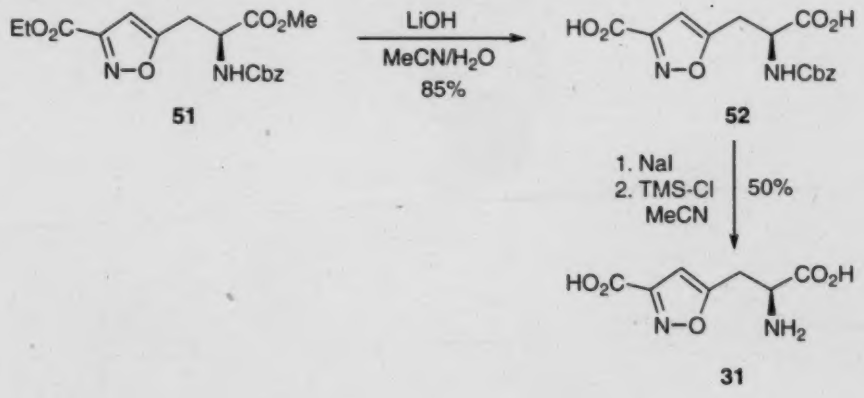
Reaction of allylglycine derivative **34** with oxime **46** (Scheme 14) gives isoxazolines **48** and **49** in 33% and 36% yield, respectively, with a trace amount of **47** (2%). Oxidation of **48** and **49** with γ-manganese dioxide as described previously affords side-product **50** (5%) and desired isoxazoline **51** (15%).

Scheme 14. 1,3-Dipolar cycloaddition reaction of **34** with nitrile oxide **46**.



Subsequent hydrolysis of **51** with lithium hydroxide provides diacid **52** in 85% yield (Scheme 15). Removal of the benzyloxycarbonyl group is then achieved using trimethylsilylchloride and sodium iodide, affording the desired DAP analogue **31** in 50% yield.

Scheme 15. Synthesis of isoxazole **31** from intermediate **51**.



1.2 Enzyme Inhibition Studies with Isoxazole Analogue 31

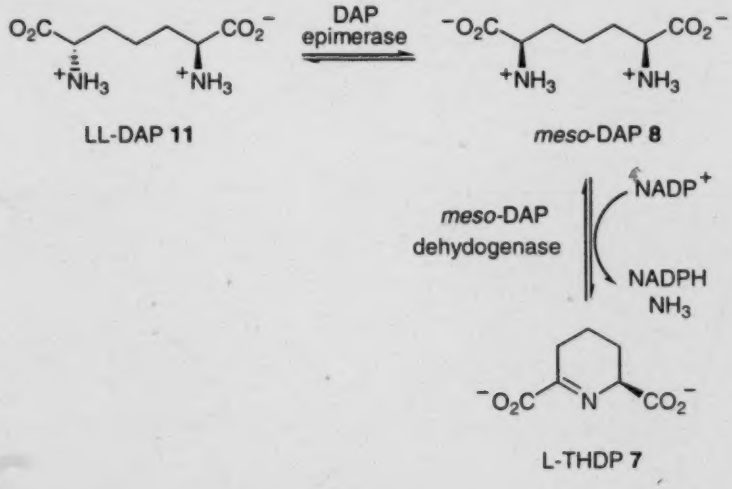
1.2.1 Inhibition Studies with DAP Epimerase and *meso*-DAP D-dehydrogenase

On completion of the synthesis of isoxazole **31**, *meso*-DAP D-dehydrogenase was purified from *Bacillus spaericus* IFO 3525 as previously reported.[50,52] In addition, DAP epimerase was isolated from an *E. coli* mutant BL21(DE3) pLysS (Strain engineered by Bruce Malcolm, Schering Plough) using a modified procedure of Wiseman and

coworkers,[58,111] to provide the enzyme as one major band on SDS page gel electrophoresis with a specific activity of 75 U/mg (Figure 20).

The *meso*-DAP D-dehydrogenase assay at pH 7.8 employs the reverse reaction, wherein $NADP^+$ oxidatively deaminates the D-amino acid center of *meso*-DAP **8** to L-THDP **7** with generation of NADPH, thereby allowing continuous spectrophotometric assay at 340 nm[52,111] (Scheme 16).

Scheme 16. Spectrophotometric assay for DAP dehydrogenase and epimerase activity.



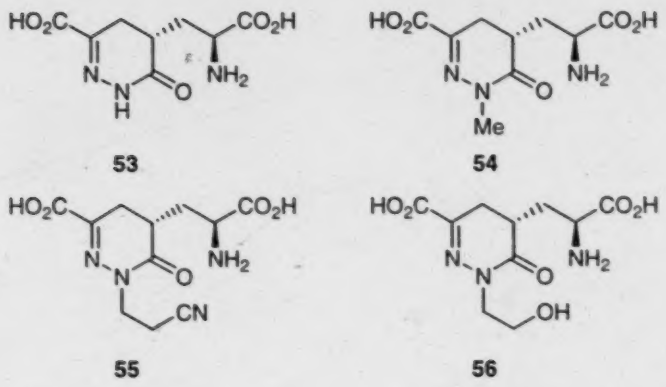
Isoxazole **31** was tested as both a substrate and an inhibitor for *meso*-DAP dehydrogenase, as described in the experimental section. Unfortunately, DAP derivative **31** is not a substrate for *meso*-DAP D-dehydrogenase and does not display any significant inhibition even at millimolar concentrations. These results further suggest that removal of the (*S*)-stereocenter from the isoxazoline ring of **15** suppresses the potency of inhibition against *meso*-DAP D-dehydrogenase. Isoxazole **31** is a flat rigid molecule that does not

possess much flexibility. This prevents strong binding in the dehydrogenase active site as compared to derivative **15** which takes on a bent conformation in the catalytic core of the enzyme (Figure 5).

Inhibition studies with DAP epimerase involve a coupled enzyme assay with *meso*-DAP dehydrogenase at pH 7.8. *meso*-DAP **8** generated by the epimerase from LL-DAP **11** is transformed by *meso*-DAP D-dehydrogenase to produce L-THDP **7** and NADPH, which is followed spectrophotometrically[111] (Scheme 16). Heterocycle **31** was first tested as a substrate for the epimerase, as described in the experimental section. However, isoxazole **31** is not accepted as a substrate or an inhibitor by the DAP epimerase enzyme. This failure of isoxazole **31** to inhibit DAP epimerase may be due to the conformational rigidity imparted by the aromatic core. Lack of inhibition may thus reflect the conformational requirements of the epimerase enzyme to bind the flexible DAP substrate in its active site, which cannot be accommodated by the isoxazole ring.

As an extension of our work on heterocyclic analogues, cyclic DAP derivatives recently synthesized by the research group of Professor Douglas Young[112] (Figure 8) were tested in our laboratory for inhibition against *meso*-DAP D-dehydrogenase and DAP epimerase. Since heterocycle **15** is a potent competitive inhibitor of *meso*-DAP D-dehydrogenase with respect to L-THDP **7**,[52] new cyclic DAP mimics possessing an (*S*)-configuration at the point of attachment of the amino acid side chain to the ring may function similarly.

Figure 8. Heterocyclic DAP mimics.



The results of enzyme inhibition tests with compounds **53-56**, summarized in Table 2, reveal that, although these cyclic mimics of L-THDP **7** possess an (*S*) configuration at the ring, all are weak inhibitors of *meso*-DAP D-dehydrogenase. The presence of an amide functionality in the 6-membered ring of these compound may create unfavorable steric interactions with the enzyme and thus diminish the degree of binding in the active site of the dehydrogenase enzyme.

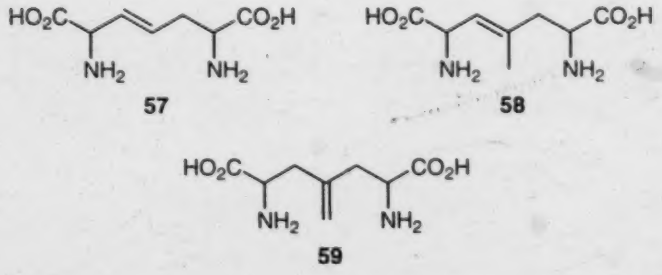
Table 2. Inhibition results for heterocyclic DAP mimics depicted in Figure 8.

DAP Analogue	*meso*-DAP D-Dehydrogenase	DAP Epimerase
53	IC_{50} 4.7 mM	IC_{50} 4.2 mM
54	No Inhibition	IC_{50} 5.4 mM
55	IC_{50} 4.7 mM	IC_{50} 2.0 mM
56	25% Inhibition at 5 mM	IC_{50} 4.3 mM

1.2.2 Inhibition studies with DAP Decarboxylase

Relatively few inhibitors have been reported for DAP decarboxylase due to the narrow substrate specificity of the enzyme. Neither L,L- nor D,D-DAP are inhibitors of the reaction.[113] *N*-hydroxy-DAP **26** and *N*-amino-DAP **27** are competitive inhibitors of the *B. sphaericus* enzyme with inhibition constants (K_i) of 84 μM and 100 μM, respectively.[114] In addition, a mixture of stereoisomers of unsaturated substrate analogues **57** (K_i 180 μM) are only moderate inhibitors of the DAP decarboxylase enzyme isolated from *E. coli* (Figure 9).[115] Other olefinic DAP derivatives such as the γ-methyl analogue **58** and γ-methylene-DAP **59** are poor inhibitors of the enzyme.

Figure 9. Olefinic DAP derivatives tested against DAP decarboxylase.

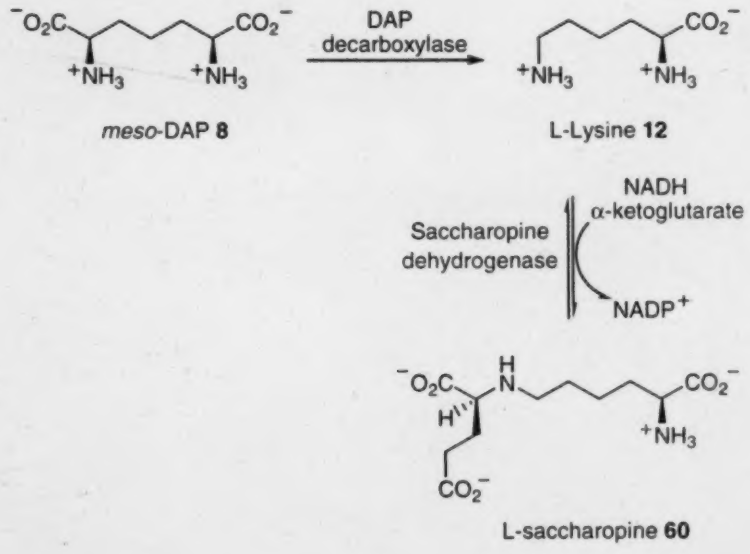


Isoxazole **31** was tested for inhibitory activity against *meso*-DAP decarboxylase, which can be purified from *Bacillus spaericus* IFO 3525 using a modified procedure,[116,117] as described in the experimental section (Figures 21, 22 and Table 3). DAP decarboxylase activity is monitored using the commercially available saccharopine

dehydrogenase in a spectrophotometric assay.[118] In the assay, the lysine formed by decarboxylation of *meso*-DAP is converted to L-saccharopine **60** with the concomitant oxidation of NADH, which is monitored as a decrease in absorbance at 340 nm (Scheme 17).

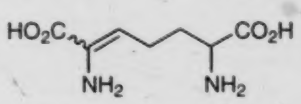
Unfortunately, heterocycle **31** fails to exhibit any inhibition against the enzyme. Although the exact reasons for the lack of inhibition against DAP decarboxylase are unknown, it is likely that the specific structural requirements of the enzyme prohibit strong binding of isoxazole **31**.

Scheme 17. Spectrophotometric assay for DAP decarboxylase activity.

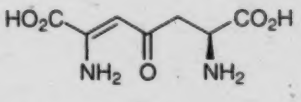


Another possible inhibitor of *meso*-DAP dehydrogenase, DAP epimerase and DAP decarboxylase, could be vinylogous amide **61**, which mimics enamine **20**.

Vinylogous amide **61** is a highly functionalized compound, containing all the structural fragments (primary amine, enamine) of enamine **20**. The ketone present in **61** could serve to stabilize the primary enamine through conjugation (in a vinylogous amide system), thereby potentially preventing the cyclization reaction, as witnessed for enamine **20** to give L-THDP **7**.



20



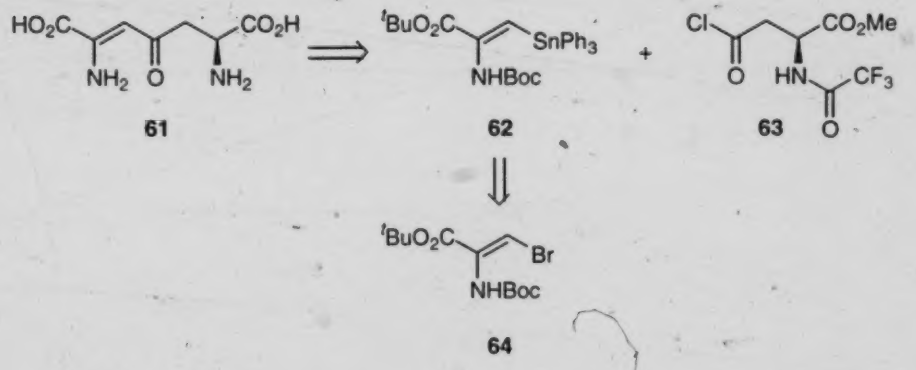
61

1.3 Synthesis of Vinylogous Amide DAP Analogue 61

1.3.1 Palladium Catalyzed Organotin Coupling

Retrosynthetic analysis of compound **61** (Scheme 18) reveals that its preparation may be realized by the coupling of an acyl chloride to a transmetallated vinyl bromide. The cross-coupling of organotin reagents with numerous electrophiles, catalyzed by palladium, provides an efficient method for the formation of a carbon-carbon bond.[119] The preparation of ketones from acid chlorides and alkylstannanes is general to both coupling partners. Hence, a wide variety of functional groups are tolerated, including ester, aldehyde, nitrile, amide and methoxy groups.[119]

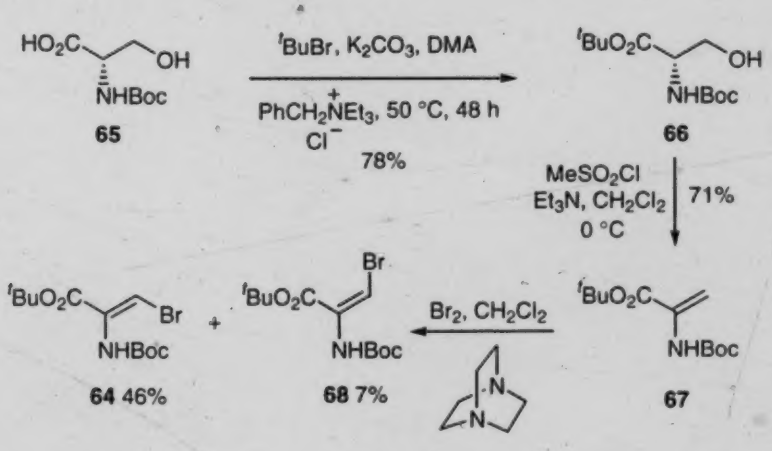
Scheme 18. Retrosynthesis of vinylogous amide **61** involving organotin coupling.



A key step in the synthesis outlined in Scheme 18 involves the formation of vinylstannane **62** from vinyl bromide **64**. Typically, hydrostannation of alkyne derivatives is often a convenient method for preparing vinylstannanes.[120] However, *cis*- and *trans*-β-tributylstannylacrylates are available by the highly stereoselective addition of tributylstannyllithium[121] to a variety of β-halo acrylates in the presence of one equivalent of copper (I) iodide.[122]

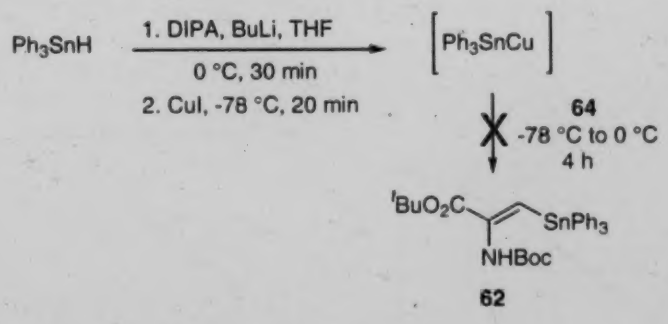
Protection of *N-tert*-butoxycarbonyl L-serine (**65**) as the *tert*-butyl ester using *tert*-butyl bromide and potassium carbonate, in the presence of a phase-transfer catalyst gives **66** in 78% yield (Scheme 19). Treatment of ester **66** with triethylamine and methanesulfonyl chloride[123] provides the dehydroalanyl derivative **67**. Following the procedure of Kishi[124] to synthesize β-halo acrylates, **67** is reacted with bromine in the presence of DABCO to furnish the desired (*Z*)-vinyl bromide **64** (46%) and a small amount of the (*E*)-vinyl bromide **68** (7%).

Scheme 19. Synthesis of vinylbromide **64** from serine derivative **65**.



Unfortunately, reaction of vinyl bromide **64** with triphenylstannyl cuprate produced *in situ* from triphenylstannyllithium does not provide the desired vinylstannane **62** (Scheme 20). Therefore, the use of tin was abandoned and our efforts were directed toward organozinc chemistry.

Scheme 20. Reaction of vinyl bromide **64** with triphenylstannyl cuprate.



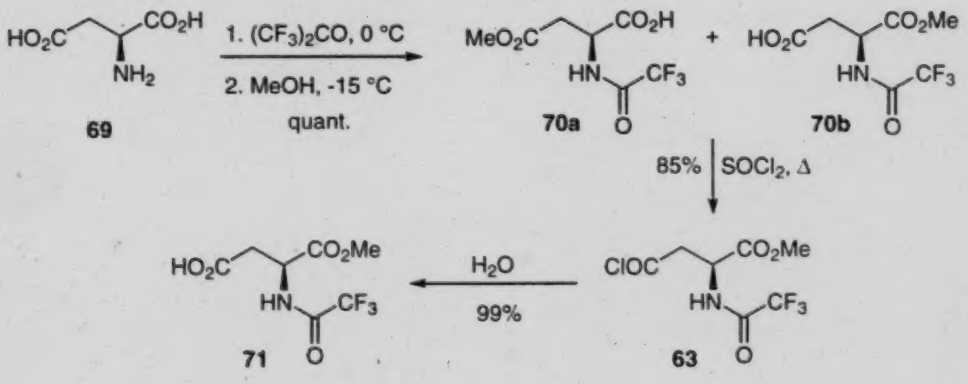
1.3.2 Palladium Catalyzed Organozinc Coupling

Recently, considerable attention has focused on the development of zinc chemistry and its synthetic applications.[125,126] Organozinc reagents tolerate a broad range of functionalities,[125,127,128] and since the introduction of highly reactive zinc, many organozinc compounds can be prepared from the corresponding organic bromide.[129,130,131] Highly reactive zinc, prepared by the lithium napthalenide reduction of zinc (II) chloride, has been shown to undergo oxidative addition to alkyl, aryl and vinyl halides under mild conditions to generate the corresponding organozinc compounds.[129]

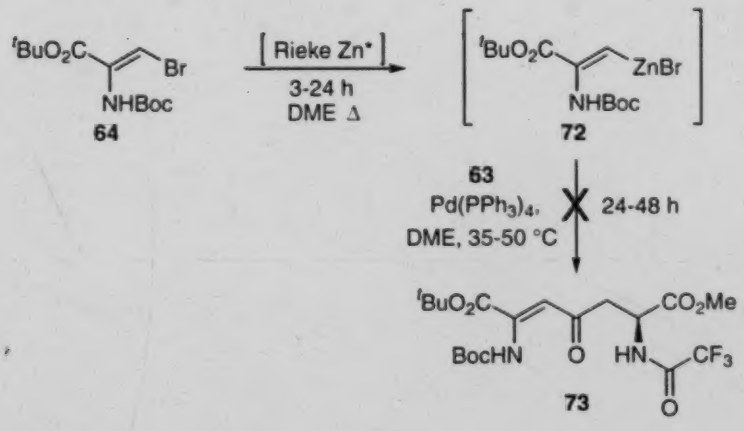
The required acid chloride **63** can be synthesized according to the literature procedure,[132] as outlined in Scheme 21. L-aspartic acid (**69**) is reacted with trifluoroacetic anhydride, and then treated with methanol to furnish a mixture of α and β-monoesters **70a** and **70b**. Treatment of this mixture with thionyl chloride under reflux provides a mixture of α and β-acid chlorides, which is recrystallized from benzene to provide crystals of the β-chloro derivative **63**, leaving the α-isomer in solution. This sensitive acid chloride is then converted to the corresponding carboxylic acid **71** by reaction with water for spectroscopic analysis.

An alternative retrosynthetic path using organozinc chemistry, similar to that outlined in Scheme 18, was then investigated as a possible method to generate DAP derivative **61**. An attempt was made to synthesize the vinylogous amide skeleton by forming zinc bromide **72** using Rieke zinc,[129] followed by palladium (0) catalyzed coupling with acid chloride **63** (Scheme 22).

Scheme 21. Synthesis of acid chloride **63** from L-aspartic acid.



Scheme 22. Attempted coupling of vinylzinc bromide **72** with acid chloride **63**.



Unfortunately, the formation of product **73** could not be detected by proton NMR or mass spectrometry. Attempts to generate this β-enamino ketone functionality using both THF

and DME as solvents, altering reaction times, and changing reaction temperatures also failed.

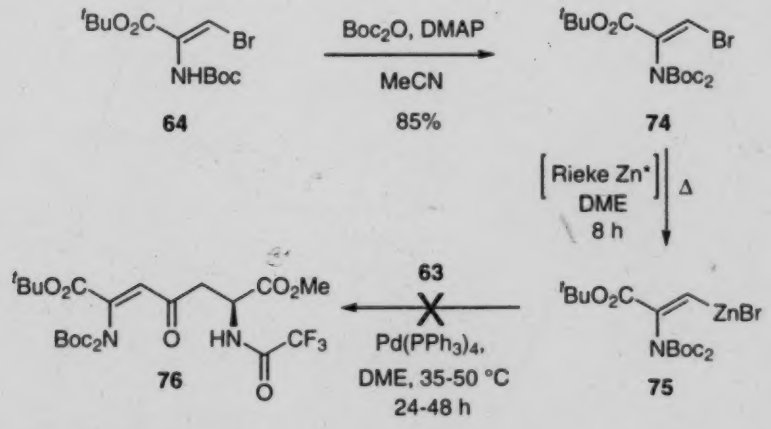
Previous work involving cross-coupling with acid chlorides demonstrated that excess zinc must be removed from the organozinc reagent solution to prevent homocoupling of the acid chlorides.[133] Precautions were taken to ensure no excess zinc was present in the solution before the addition of the acid chloride **63**. However, careful analysis of the reaction mixture showed that no homocoupled side-product of the acid chloride had formed. If the organozinc bromide **72** is indeed formed, quenching of the reaction should produce the corresponding dehydroalanyl derivative **67**. However, none of this product could be observed by TLC.

It seemed that perhaps the acidic amide proton could interfere with the reaction and possibly quench the organozinc reagent. Hence, vinylbromide **64** was transformed to the corresponding di-protected derivative **74** as outlined in Scheme 23. As before, reaction of **72** with Rieke zinc followed by palladium catalyzed coupling with acid chloride **63** fails to give the desired vinylogous amide **76**.

Recent mechanistic investigations of the reaction of highly reactive zinc with organic bromides[134] confirm that the oxidative addition process shows pronounced structure-reactivity dependence, in contrast to that shown by some other metals. For instance, comparison of the reactivity of bromobenzene and 1-brompentane reveals that it reacts with active zinc 27 times slower than the primary alkyl bromide. In addition, competitive reaction of vinylic bromide 2-bromo-3-methyl-2-butene and bromobenzene with active zinc in THF at 70 °C gives a rate ratio of 0.017, corresponding to a rate *160*

times slower than 1-bromopentane. The rate limiting step may thus be the formation of the vinyl zinc bromide.

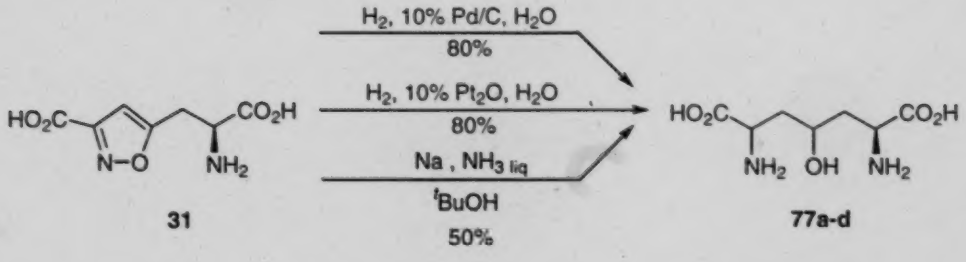
Scheme 23. Attempted coupling of vinylzinc bromide **74** with acid chloride **63**.



1.3.3 Reductive Ring Opening of Isoxazolines

From a new retrosynthetic perspective, it seemed that the vinylogous amide functionality could be achieved by reductive ring opening of the previously synthesized isoxazole **31**.[135] It is well known that isoxazole derivatives play a variety of roles in the generation of new molecular frameworks[136] and the nitrogen-oxygen bond can be cleaved under a variety of conditions. However, attempts to transform the isoxazole ring into the target vinylogous amide using standard conditions, such as hydrogenation[135,137,138] or reduction with sodium in liquid ammonia[135] produce exclusively the saturated alcohol **77a-d** as a mixture of four diastereoisomers (Scheme 24).

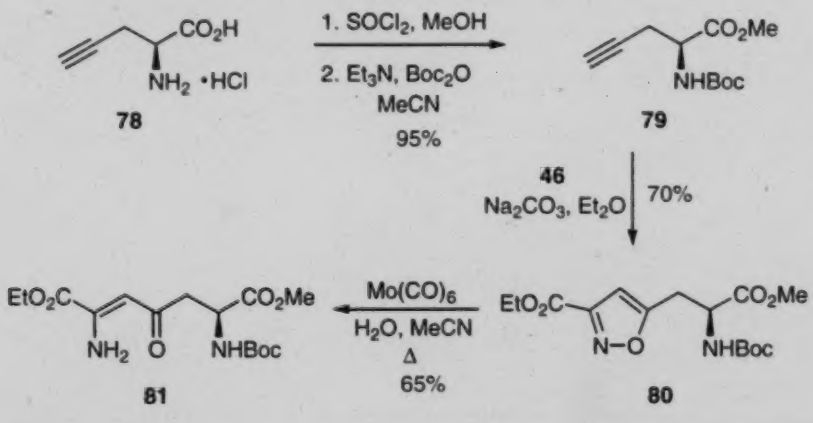
Scheme 24. Reduction of isoxazole **31** to saturated alcohol **77a-d**.



Further investigations into this reductive ring opening required additional work to improve the synthesis of **31**. It is known that isoxazole moieties can be generated by cycloaddition reaction between propargyl derivatives and nitrile oxides.[139] As L-propargyl glycine (**78**) became commercially available, it seemed that a cycloaddition reaction between chloro oxime **46** and the corresponding propargyl derivative would afford the isoxazole moiety in one step. Protection of L-propargyl glycine (**78**) using standard protocols gives methyl *N*-(*tert*-butoxycarbonyl)-L-propargyl glycinate (**79**)[140] in 95% yield (Scheme 25).

Treatment of **79** with ethyl chlorooximidoacetate **46** under basic conditions affords the 1,3-dipolar cycloaddition product, isoxazole **80**, as a single regioisomer in 70% yield. Formation of the vinylogous amide moiety is realized by treatment of isoxazole **80** with a catalytic amount of molybdenum hexacarbonyl and one equivalent of water in refluxing acetonitrile[141] to afford **81** (65%).

Scheme 25. Synthesis of vinylogous amide **81** from L-propargyl glycinate **78**.

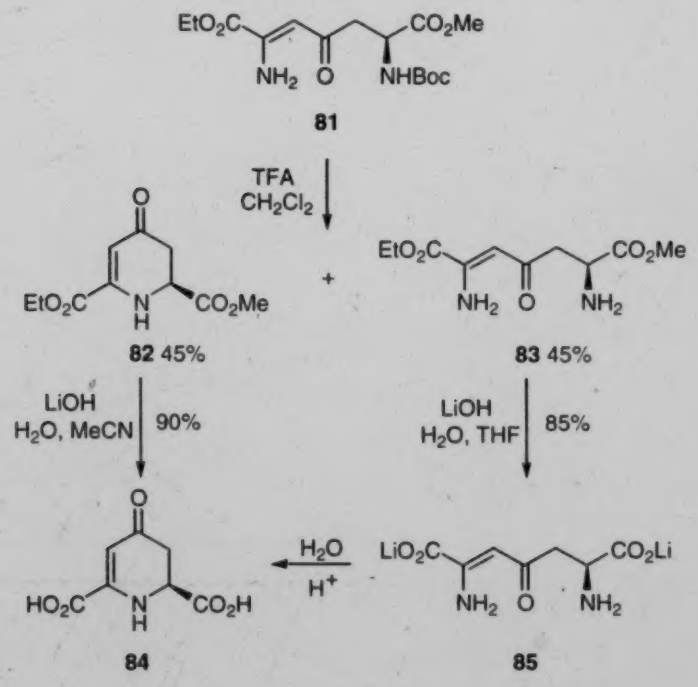


The structure of **81** is confirmed by the presence of a ketone in the ^{13}C NMR spectrum. Furthermore, an upfield shift of the olefinic hydrogen from 6.42 ppm in the isoxazole to 5.85 ppm in the vinylogous amide is also observed in the ^{1}H NMR spectrum. Although double bond isomerization of **81** was considered, only one isomer is detected by NMR experiments. Subsequent exposure of **81** to excess trifluoroacetic acid (TFA) in dichloromethane for one hour generates a 1:1 mixture of cyclic and open-chain vinylogous amides **82** (45%) and **83** (45%) (Scheme 26). The cyclic derivative **82** could arise from Michael addition of the nitrogen liberated by removal of the *tert*-butoxycarbonyl moiety onto the α,β-unsaturated alkene followed by subsequent loss of ammonia (Scheme 27). The yield of **83** can be improved to 80% by using five equivalents of TFA and careful monitoring of the reaction by TLC.

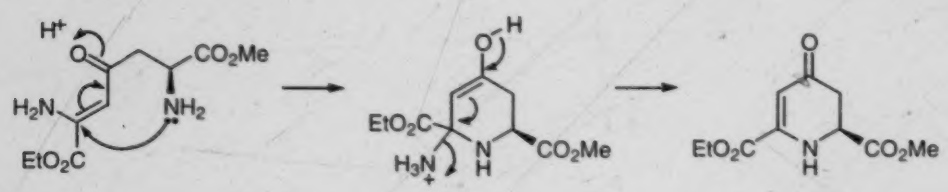
Hydrolysis of di-ester **82** using lithium hydroxide followed by work-up gives **84** in 90% yield. The proton NMR spectrum of compound **84** reveals that the olefinic proton

is exchangeable with deuterium. Enolization of the vinylogous amide is a likely cause for this observation. Compound **83** is unstable at room temperature and is therefore transformed immediately into the di-lithium salt **85** (85% yield). Acidification of **85** leads rapidly to formation of the cyclized derivative **84**, hence this compound is stored as its di-lithium derivative. Unlike the cylic vinylogous amide, the olefinic proton of **85** is not exchangable with deuterium in the presence of deuterated solvents.

Scheme 26. Synthesis of vinylogous amides **84** and **85**.



Scheme 27. Proposed mechanism for the formation of cyclic vinylogous amide **82**.



83 **86** **82**

Cyclic vinylogous amide **84** possesses a structure that is similar to an alkaloid known as betanidin. With an efficient synthesis for compound **82**, the total synthesis of this natural product was attempted. The results of these synthetic investigations are discussed in the final section of this chapter.

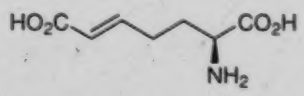
1.4 Enzyme Inhibition Studies with Vinylogous Amides

1.4.1 Inhibition Studies with *meso*-DAP Dehydrogenase and DAP Epimerase

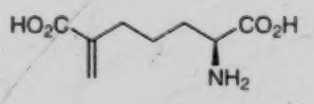
Compounds **84** and **85** are not substrates for *meso*-DAP D-dehydrogenase and show only poor reversible inhibition of *meso*-DAP D-dehydrogenase, with IC_{50} values in the range of 0.4-0.45 mM. This is surprisingly weak binding given that these molecules possess much of the functionality present in the substrates (L-THDP **7** and *meso*-DAP **8**) and putative imine intermediate **14**. Although the presence of a ketone at the center of **85** could generate unfavorable steric interactions with the enzyme, a more likely cause for

failure of effective binding to *meso*-DAP dehydrogenase is the co-planarity of the vinylogous amide system.

Interestingly, unsaturated α-aminopimelic acids **87** and **88** were recently shown to be reversible inhibitors of *meso*-DAP dehydrogenase with K_i values of 5.3 μM (competitive) and 44 μM (non-competitive) respectively.[142] Crystallographic studies reveal that the isoxazoline **15** and the unsaturated analogue **88** bind in the active site of *meso*-DAP D–dehydrogenase with conformations for which analogues **84** and **85** would probably require bond rotation in the co-planar portions.[57,143]



87

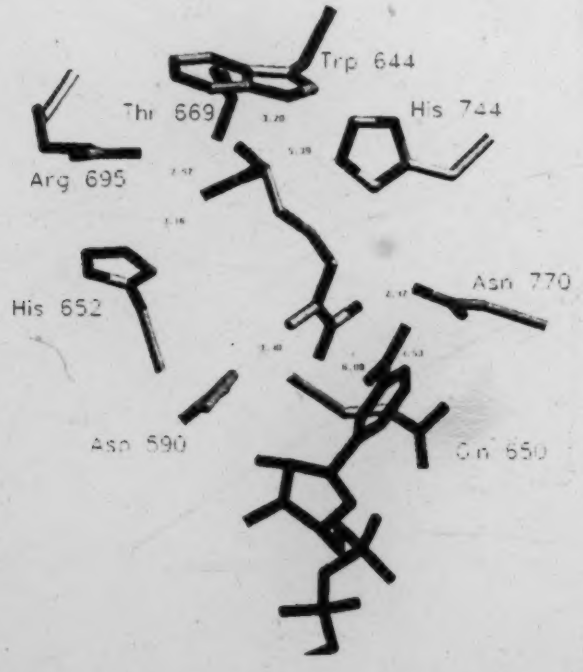


88

The three-dimensional structure of *C. glutamicum meso*-DAP dehydrogenase in a ternary complex with NADPH and L-2-amino-6-methylene-pimelate (**88**) offers a structural rationale for the potency of inhibition exhibited by this olefinic DAP derivative[143] (Figure 10). Although, the replacement of the α-amino group of the D-stereocenter of *meso*-DAP **8** with the unsaturated methylene group eliminates the hydrogen bonding interactions with Asp590 and Asp620, the interactions between the carboxylate of **88** and both Asn770 and Gly651 are maximized. Furthermore, the α-amino group of the unsaturated inhibitor participates in an end-on aromatic-amine hydrogen bond with the indole ring of Trp644. The above enhanced enzyme-inhibitor

interactions reveal that a basic nitrogen at the reactive D-stereocenter of *meso*-DAP **8** may not be required for potent inhibition of the *meso*-DAP D-dehydrogenase enzyme.

Figure 10. Close up view of inhibitor binding site of *C. glutamicum* DAP dehydrogenase, with L-2-amino-6-methylene-pimelate (**88**) bound at the active site.



Enzymatic results show that, as expected, cyclic analogue **84** is a very poor inhibitor of DAP epimerase. Disappointingly, the acyclic vinylogous amide **85** is also a weak reversible inhibitor (IC_{50} of 0.5 mM) of this enzyme despite containing most of its atoms in locations that might be expected to mimic the α-anion transition state. The crystal structure of active DAP epimerase with a substrate analogue in the active site is not yet available, and therefore rationalization as to why these compounds are weak

inhibitors is difficult. However, it is likely that the poor binding to DAP epimerase may be again due to the planarity of the vinylogous amide system as well as the consequent reduced basicity of the amino group attached to the sp^2 carbon. Initially it was proposed that enzyme-induced tautomerization of **85** to its tautomer **85a** could trigger addition of an active site thiol group, but the required tautomerization has not been observed (Scheme 28) by proton or carbon-13 NMR experiments.

Scheme 28. Potential enzyme-induced tautomerization of **85** to its tautomer **85a**.

85 **85a**

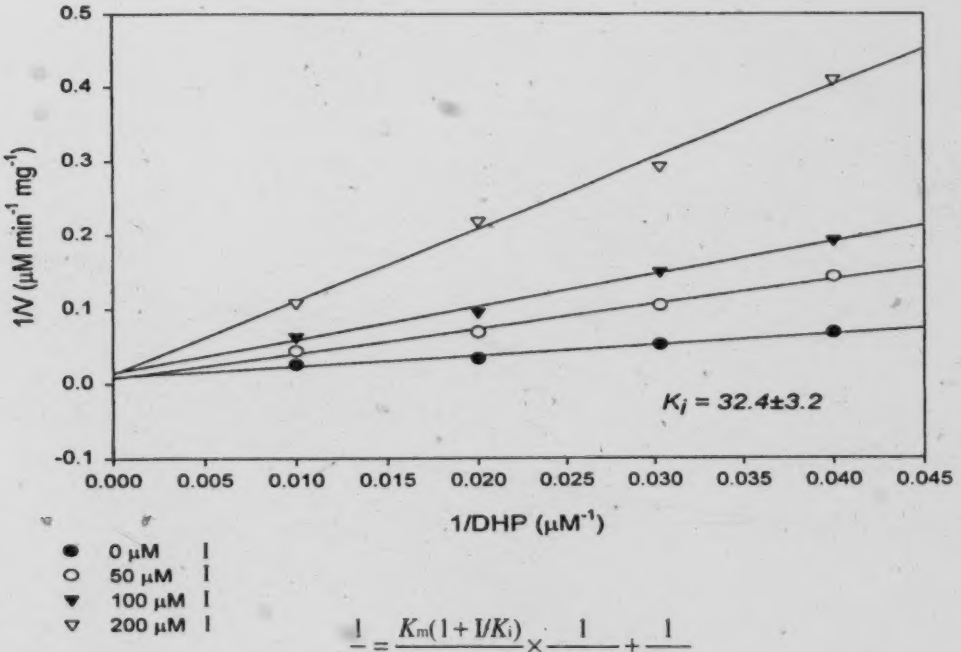
1.4.2 Inhibition Studies with L-DHDP Reductase

The cyclic vinylogous amide **84**, which shows considerable structural similarity to L-DHDP **6**, proves to be a good inhibitor of L-DHDP reductase.[144] Inhibition studies, performed by Renjian Zheng of Professor J. S. Blanchard's group as described in the experimental section, demonstrate that **84** is a reversible competitive inhibitor of the DHDP reductase with respect to L-DHDP with an inhibition constant (K_i) of 32 μM

(Figure 11). Hence **84** is comparable to the most potent competitive inhibitor of DHDP reductase reported thus far, the fully planar dipicolinic acid (**13**) (K_i 26 μM).

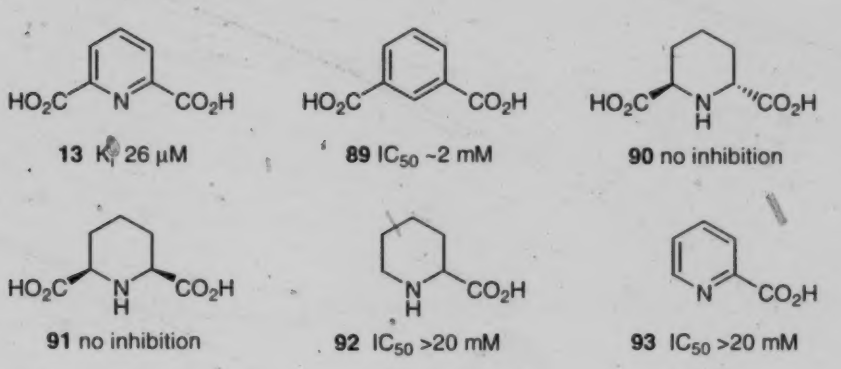
Since related derivatives such as isophthalic acid (**89**), a racemic mixture of *trans*-piperidine dicarboxylic acids (**90**), *cis*-piperidine dicarboxylic acid (**91**), pipecolic acid (**92**) and picolinic acid (**93**) are poor inhibitors[26,30] (Figure 12), additional crystallographic studies of **84** bound to DHDP reductase should help clarify the substrate-enzyme interactions at the stereogenic center of DHDP. This could potentially lead to other cyclic derivatives with even greater inhibition.

Figure 11. Plot for determination of K_i for analogue **84**.



$$\frac{1}{v} = \frac{K_m(1 + I/K_i)}{V_{max}} \times \frac{1}{[DHP]} + \frac{1}{V_{max}}$$

Figure 12. DHDP analogues tested against L-DHDP reductase.

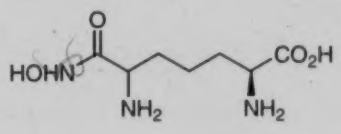


1.4.3 Inhibition studies with *meso*-DAP Decarboxylase

Vinylogous amides **84** and **85** were tested as substrates and inhibitors for DAP decarboxylase. Since these molecules exhibit UV absorption maxima at 340 nm, the spectrophotometric assay employing saccharopine dehydrogenase which monitors decrease in NADH concentration at 340 nm (Scheme 17) could not be used. Therefore, DAP decarboxylase activity was estimated by measuring the $^{14}CO_2$ evolution from [1,7-^{14}C]-DAP as described by Kelland *et al.*[145] and detailed in the experimental section. Unfortunately, both **84** and **85** fail to show any inhibitory activity against the enzyme. Although cyclic derivative **84** would not be expected to inhibit DAP decarboxylase, the failure of **85** is probably due to the narrow substrate specificity of the enzyme and reduced basicity of the nitrogen. The presence of the ketone in the vinylogous amides

may also be introducing unfavourable steric interactions in the enzyme active site thereby preventing effective binding.

In an attempt to further explore the substrate specificity of the *meso*-DAP dehydrogenase, epimerase and decarboxylase enzymes, a new amino acid derivative, diastereomeric mixture **94ab** which possesses all the structural features of DAP but with a hydroxamic acid functionality was chosen. Hydroxamate moieties closely resemble carboxylic acids, thus DAP derivative **94ab** was proposed to be a substrate of the DAP enzymes. Furthermore, it may be that replacement of the carboxylic acid group at the D-center of *meso*-DAP with a hydroxamic acid moiety will lead to inhibition of DAP decarboxylase as PLP-assisted cleavage of the carbon-nitrogen hydroxamate bond is unlikely.



94ab

2. Hydroxamic Acid Analogues as Enzyme Inhibitors

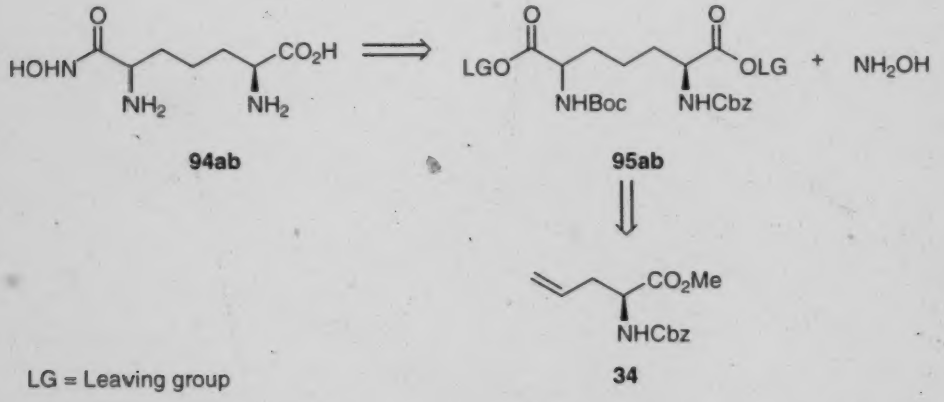
2.1 Synthesis of DAP Hydroxamic Acid 94ab

The hydroxamic acid functionality is a key constituent of many biologically relevant compounds, such as anticancer, antibacterial and antifungal agents.[146,147,148] Hydroxamates are extremely effective metal-ion chelators and potent inhibitors of matrix metalloprotease enzymes.[149,150,151] In addition, serine hydroxamate has antibacterial activity

against *E. coli* whereas lysine hydroxamate reduces the rate of *E. coli* growth.[152] Several commonly used methods for the synthesis of hydroxamic acids include the direct acylation of hydroxylamine,[153] or its protected derivatives such as *O*-benzyl[154] or *N,O*-tris-trimethylsilyl[155] hydroxylamine, with various acylating agents such as carboxylic acid chlorides or activated esters. In addition, solid phase synthesis of hydroxamic acids is possible.[156,157]

A retrosynthetic path to target **94ab**, presented in Scheme 29, uses intermediate **34** as the building block for the DAP skeleton. Once the main chain is constructed, activation of the DAP analogue can produce intermediate **95ab**. Attack by hydroxylamine at the activated esters may provide a separable mixture of two mono-hydroxamic acids.

Scheme 29. Retrosynthesis of mono-hydroxamic acid of **94ab**.

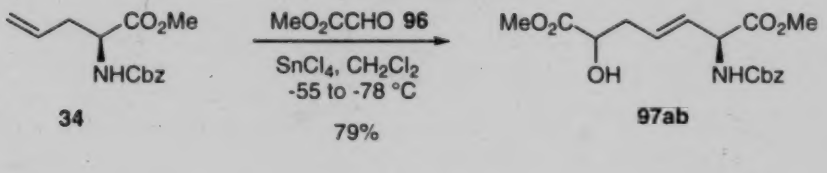


94ab **95ab** **34**

LG = Leaving group

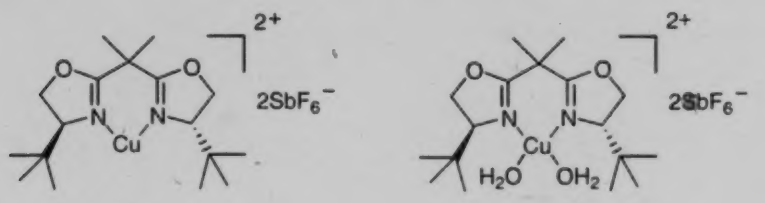
The DAP framework is readily generated using a modified procedure of Cox *et al.*[158] (Scheme 30). Ene reaction of methyl glyoxylate **96**, synthesized according to the literature procedure,[159] and allyl glycine derivative **34** in the presence of tin (IV) chloride, provides olefin **97ab** as a mixture of two diastereoisomers (1:1) in good yield (79%).

Scheme 30. Ene reaction of methyl glyoxylate **96** and allyl glycine derivative **34**.



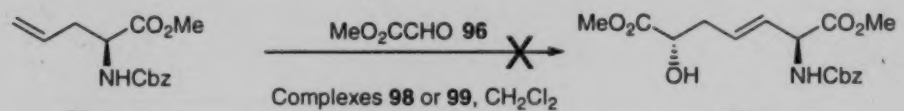
Major advances in the development of chiral Lewis Acid catalyzed ene reactions have been reported.[160] In most cases, the reactions fail or give little stereoselectivity with mono-substituted olefins. However, Evans and co-workers have reported that bidentate bis(oxazolinyl) (box) Cu (II) complexes **98** and **99**, which are effective enantioselective catalysts in Diels-Alder,[161] aldol,[162] and Michael reactions[163] (Figure 13), catalyze the enantioselective condensation of a variety of olefins (including mono-substituted) to glyoxylate esters to provide α-hydroxy esters.[164] Complexes **98** and **99** can be prepared according to a literature procedure[165] from the commercially available 2,2-bis[2-[4(*S*)-*tert*-butyl-1,3-oxazolinyl]]propane [(*S*,*S*)-*tert*-butylbis(oxazoline)]. Attempted conversion of **34** to the (2*S*,6*S*) alcohol **97a** using either Lewis acid copper complex in the presence of 5-10 equivalents of methyl glyoxylate fails and leads only to the recovery of starting material (Scheme 31).

Figure 13. Bis(oxazolinyl) (box) Cu (II) complexes **98** and **99**.



98 **99**

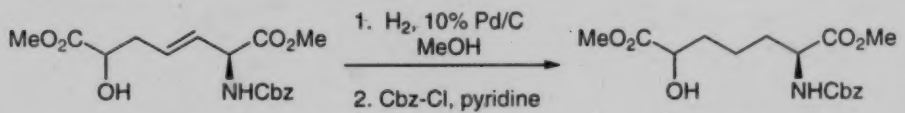
Scheme 31. Attempted conversion of **34** to the (2*S*,6*S*) alcohol **97a**.



34 **97a**

For synthetic simplicity, it was thus decided to first determine if the diastereomeric mixture of the target DAP analogue was active against the key enzymes under investigation, before exploring this chiral 'ene' reaction further. Intermediate **97ab** was thus left as a mixture and used for further transformations. Hydrogenation of intermediate **97ab** results in removal of the double bond as well as the Cbz-protecting group (Scheme 32). Reprotection of the nitrogen with benzyl chloroformate and pyridine affords the saturated alcohol **100ab** in 61% overall yield.

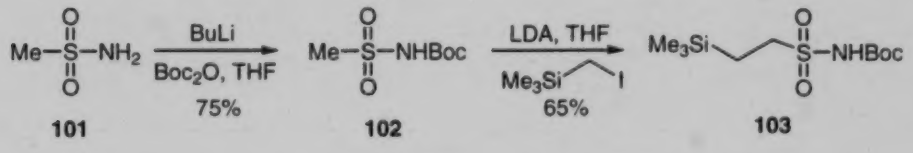
Scheme 32. Hydrogenation of intermediate **97ab**.



97ab **100ab**

The next transformation requires introduction of an amine functionality in place of a hydroxy group. The chosen strategy involves the use of the Mitsunobu reaction.[166] One major advantage of the Mitsunobu process is that it can lead to a variety of protected DAP derivatives, by virtue of the identity of the nitrogen nucleophile. The doubly protected amide **103** was chosen as the nucleophile for the Mitsunobu transformation. Treatment of methanesulfonamide **101** with *n*-butyllithium followed by di-*tert*-butyl pyrocarbonate using a modified literature procedure,[167] gives methane-Boc-sulfonamide **102** in 75% (Scheme 33). Sulfonamide **102** reacts with (iodomethyl)trimethylsilane in the presence of LDA to generate the desired carbamate **103** (65%).

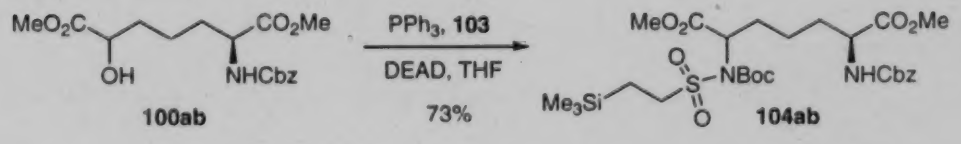
Scheme 33. Synthesis of carbamate **103**.



101 **102** **103**

Conversion of alcohol **100ab** to amide **104ab** (73%) *via* the Mitsunobu reaction with carbamate **101** proceeds in the presence of triphenylphosphine and diethylazodicarboxylate (DEAD) (Scheme 34).

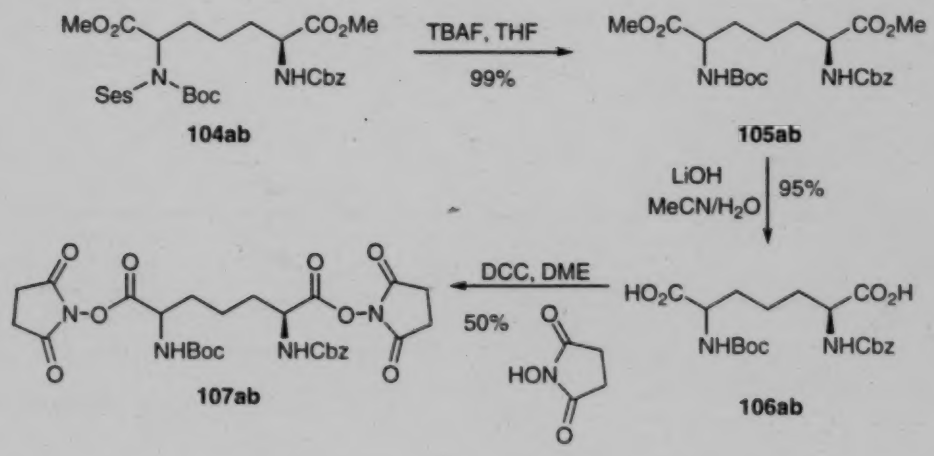
Scheme 34. Mitsunobu reaction of alcohol **100ab**.



100ab **104ab**

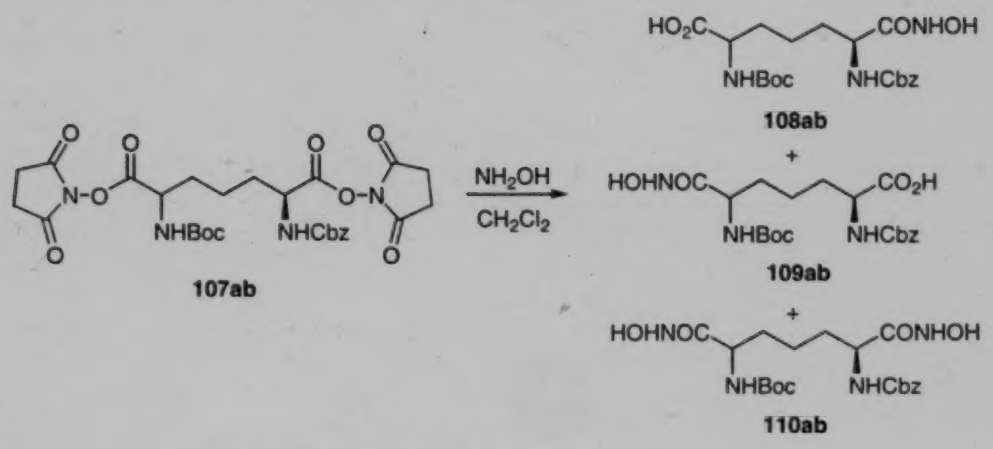
Treatment of compound **104ab** with tetrabutylammonium fluoride in THF provides diester **105ab** in 99% yield (Scheme 35). Hydrolysis of the esters with lithium hydroxide in an acetonitrile/water solution affords diacid **106ab**. Conversion of this diacid to the activated *N*-hydroxysuccinyl diester **107ab** (50%) is achieved using DCC as a coupling reagent.

Scheme 35. Synthesis of di-*N*-hydroxy succinimide derivative **107ab** from **104ab**.



Reaction between the activated DAP derivative **107ab** and hydroxylamine results in the formation of a complex diastereomeric mixture of mono- and di-hydroxamic acids **108ab**, **109ab** and **110ab**, as determined and mass spectrometry of this crude reaction mixture (Scheme 36). Since purification of this mixture of hydroxamic acids is difficult, the exact ratio of these compounds was not determined. To overcome these problems, reactions of lysine analogues were explored for insight into the reactivity of hydroxylamine with α-amino esters having *N*-Cbz or *N*-Boc groups.

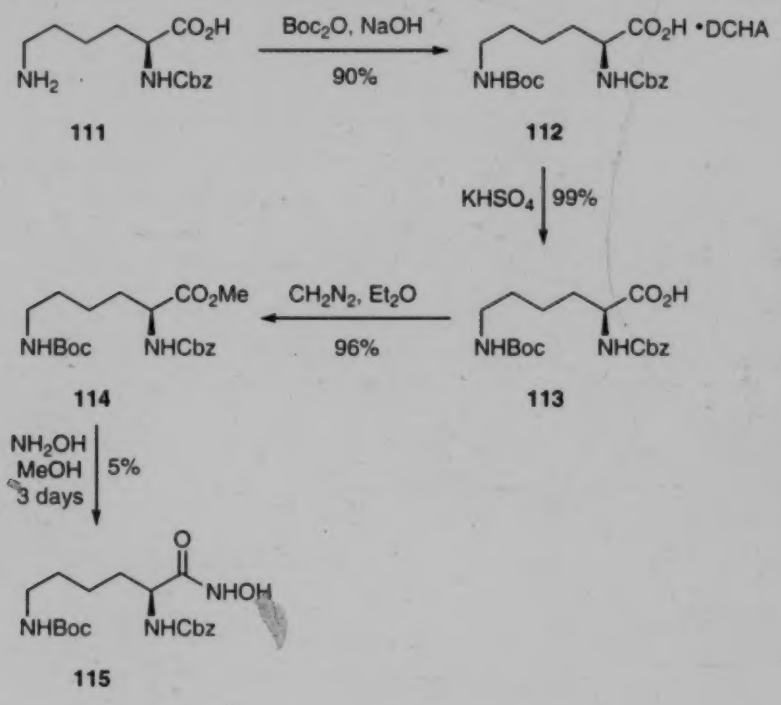
Scheme 36. Reaction of DAP derivative **107ab** with hydroxylamine.



Reaction of L-lysine derivative **111** with di-*tert*-butyl pyrocarbonate under standard conditions, followed by treatment with dicyclohexylamine (DCHA) furnishes crystals of the dicyclohexylamine salt **112**[168] (90%) as shown in Scheme 37. Addition of a 0.5 M potassium hydrogen sulfate solution affords the free acid **113**[169] in 99% yield. Esterification of intermediate **113** with diazomethane[170] gives methyl ester **114**[171] in 96% yield. At this point, the reactivity of hydroxylamine towards the methyl ester of the α-protected lysine derivative was investigated.

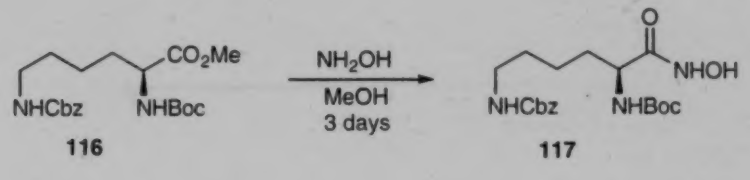
Reaction of compound **114** with a 1 M solution of hydroxylamine in methanol for 3 days, according to a modified procedure of Cohen *et. al.*,[153] affords the desired hydroxamic acid **115** in only 5% yield.

Scheme 37. Synthesis of L-lysine hydroxamic acid derivative **115**.



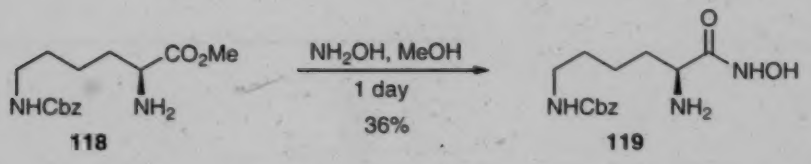
Furthermore, attempts to react the α-*N*-Boc protected lysine derivative **116**,[172] prepared by methylation of commercially available material, with hydroxylamine also provides a low yield (3-5%) of the desired hydroxamate **117** (Scheme 38), as confirmed by ^{1}H NMR and mass spectrometry.

Scheme 38. Conversion of L-lysine derivative **116** to hydroxamic acid **117**.



In order to understand if the substituent on the amino moiety is influencing the rate of nucleophilic attack at the ester carbonyl, commercially available lysine analogue **118** was reacted with hydroxylamine for one day to furnish hydroxamate **119** in 36% yield (Scheme 39). Hence, the presence of a Cbz or Boc protecting group appears to provide steric hindrance to nucleophilic displacement of the methyl ester by hydroxylamine. This methodology could thus be extended to the synthesis of target **94ab**.

Scheme 39. Conversion of L-lysine derivative **118** to hydroxamic acid **119**.

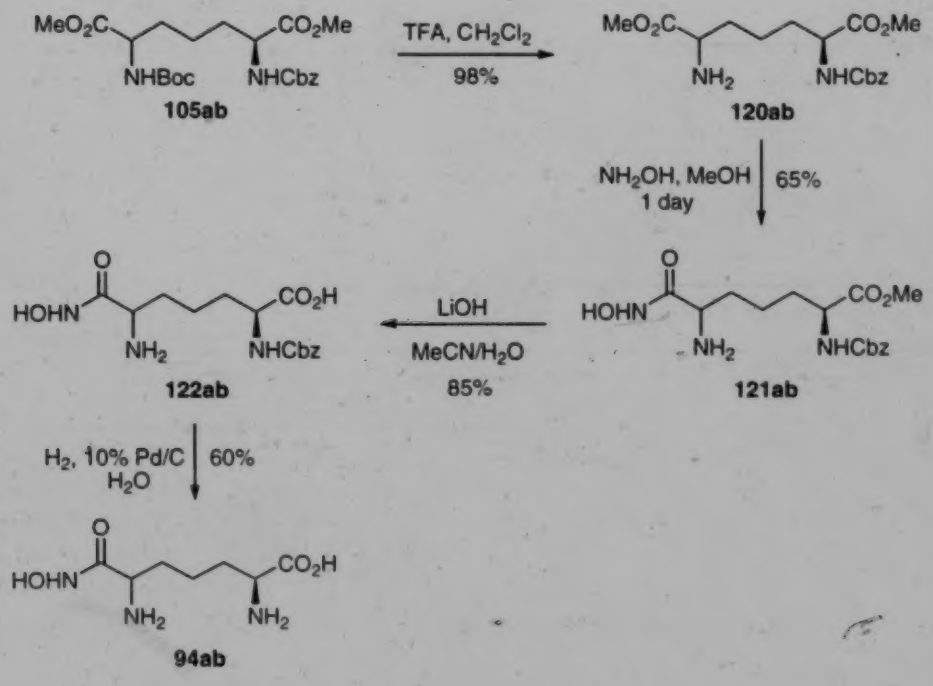


Intermediate **105ab** is readily converted to the corresponding amine **120ab** by reaction with TFA in dichloromethane (Scheme 40). Treatment of **120ab** with three equivalents of hydroxylamine in methanol for 1 day furnishes the expected hydroxamic acid **121ab** in 65% yield with recovery of starting material (25%). To prevent the formation of a dihydroxamic acid species, the reaction was stopped after one day. The identity and position of this hydroxamic acid functionality could be confirmed by the upfield shift of the α-proton signal ($C\underline{H}NH_2$) at 4.01 ppm in the starting diester **120ab** to 3.65 ppm in the product.

Hydrolysis of the methyl ester of **121ab** with lithium hydroxide affords the diacid **122ab** in 85% yield. Finally, deprotection of the amino functionality using 10% Pd/C in

water furnishes the desired DAP derivative **94ab** (60%) as a mixture of two diastereoisomers (1:1).

Scheme 40. Synthesis of hydroxamic acid analogue **94ab** from intermediate **105ab**.



2.2 Enzyme Inhibition Studies with Hydroxamic Acid Analogue 94ab

2.2.1 Inhibition Studies with *meso*-DAP Dehydrogenase and DAP Epimerase

Inhibition studies reveal that hydroxamic acid **94ab** is a substrate for *meso*-DAP dehydrogenase with a K_m value of 1.39 mM ± 0.17 mM and a V_{max} of 0.026 mM/min ± 0.002 mM/min. The hydroxamic acid is therefore an effective substrate for the dehydrogenase enzyme, comparable to its natural substrate, *meso*-DAP **8**, which has a K_m value of 2.5 mM.[80] The enzyme assay is performed at pH 7.8. Hence, the hydroxamic acid moiety should be deprotonated, thereby mimicking the carboxylate functionality and participating in critical hydrogen bond interactions with residues in the enzyme active site. Although **94ab** is a mixture of two diastereomers, the *meso*-isomer **94b** should be the compound which is accepted by the enzyme. Determination of the binding constant for the pure **94b** would therefore give a lower K_m value.

Additional experiments with DAP epimerase indicate that hydroxamic acid **94ab** is a substrate for this enzyme. Substrate tests for the epimerase are performed in deuterated buffer with inclusion of the substrate analogue. After incubation with the enzyme for 18 hours, anion exchange chromatography is employed to recover the compound and both proton and deuterium NMR spectra are obtained. If the compound being tested is a substrate for DAP epimerase, then racemization of the D-stereocenter will result in replacement of an α-hydrogen for deuterium from the buffer solution. As a control, a mixture of all possible isomers of DAP (D,D-, L,L-, D,L-) is incubated with epimerase in deuterated buffer, and after 18 hours approximately 70% of the α-hydrogens

are replaced with deuterium. Analogue **94ab** shows 10-15% deuterium incorporation at the α-center. The ^{1}H NMR spectra suggests that the proton α- to the carboxylic acid is the center being epimerized. However, due to the low incorporation of deuterium in this diastereomeric mixture and contamination from buffer constituents, a broad-band ^{13}C NMR experiment (expected to reveal a triplet at 49 ppm) did not allow unambiguous determination of the position of deuterium incorporation.

2.2.2 Inhibition Studies with DAP Decarboxylase

Inhibition tests with DAP decarboxylase indicate that the hydroxamic acid **94ab** is a weak reversible inhibitor of the enzyme with an IC_{50} value of 0.35 mM. The presence of the hydroxamic acid may be influencing the rate of decarboxylation of the natural substrate.

2.2.3 Inhibition Studies with *meso*-DAP Adding Enzyme

A family of ATP dependent amino acid ligases, MurC to MurF, is responsible for the assembly of UDP-MurNAc-L-Ala-D-Glu-*m*-DAP-D-Ala-D-Ala **123**, a cytosolic precursor in the biosynthesis of peptidoglycan in both Gram-negative and Gram-positive bacteria.[173] The *meso*-DAP-adding enzyme (MurE) is the ligase responsible for the formation of UDP-MurNAc-L-Ala-D-Glu-*m*-DAP.[174] The catalytic mechanism of the

MurE reaction is assumed to be similar to that employed by many of the well characterized ATP-dependent ligases[16,175] (Scheme 41). The reaction proceeds *via* carboxylate activation by ATP to give an acyl phosphate intermediate **125**, followed by attack of an amino group of *meso*-DAP **8** to form tetrahedral intermediate **126**. Collapse of tetrahedral intermediate **126** furnishes the tripeptide **127** that undergoes a final reaction with the D-Ala-D-Ala ligase (MurF) to form the UDP-MurNAc-pentapeptide **123**.

Several structural analogues of *meso*-DAP **8** are substrates for the enzyme,[174,176] including L,L-DAP, which has a 2000-fold higher K_m than D,L-DAP, suggesting that the enzyme is not completely enantiospecific.[177] Generally, the greatest extents of inhibition for *meso*-DAP adding enzyme are observed with the best substrates. This presumably reflects that these compounds behave as competitive substrates. Substrate analogues such as β-fluoro derivative **19**, and *N*-hydroxy-DAP **26** are substrates for *meso*-DAP adding enzyme and show weak inhibitory activity (Figure 14).[176] *Meso*-lanthionine **128** and γ-methylene-DAP **59** however, show no significant inhibition at millimolar concentrations.

Inhibition studies with hydroxamic acid **94ab**, performed by the research group of Dr. Jean van Heijenoort (Biochimie Moléculaire et Cellulaire, Université de Paris-Sud, France) reveal that this compound is a weak inhibitor of the adding enzyme with an IC_{50} value of 1 mM.

Scheme 41. Proposed catalytic mechanism of the MurE reaction.

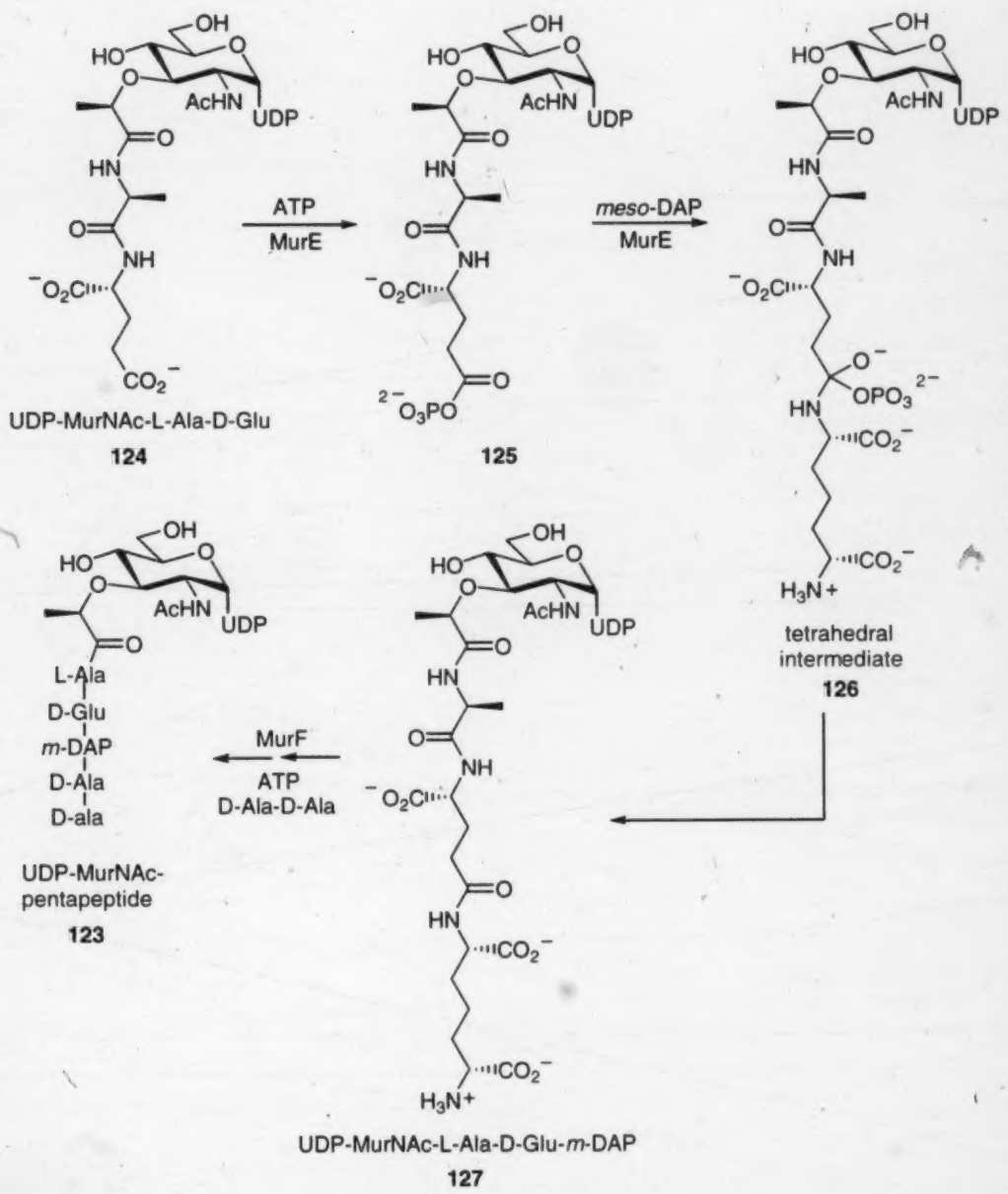
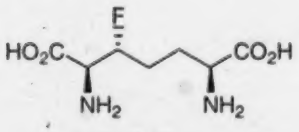


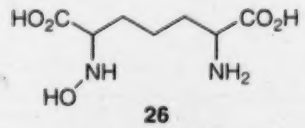
Figure 14. DAP derivatives tested against *meso*-DAP adding enzyme.



19

K_m 3.5 mM

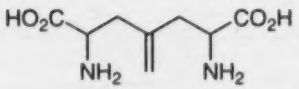
IC_{50} 2.3 mM



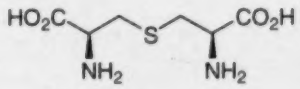
26

K_m 9.2 mM

IC_{50} 0.56 mM



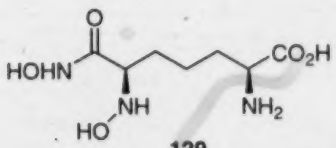
59



128

K_m 1.5 mM

Future attempts to improve the potency of inhibition of this hydroxamate could involve the stereoselective synthesis of an *N*-hydroxy DAP hydroxamic acid **129**, since the diastereomeric mixture of **26** is an alternative substrate for the adding enzyme and strongly inhibits incorporation of *meso*-[^{14}C]-DAP into the UDP-MurNAc-pentapeptide.[176]



129

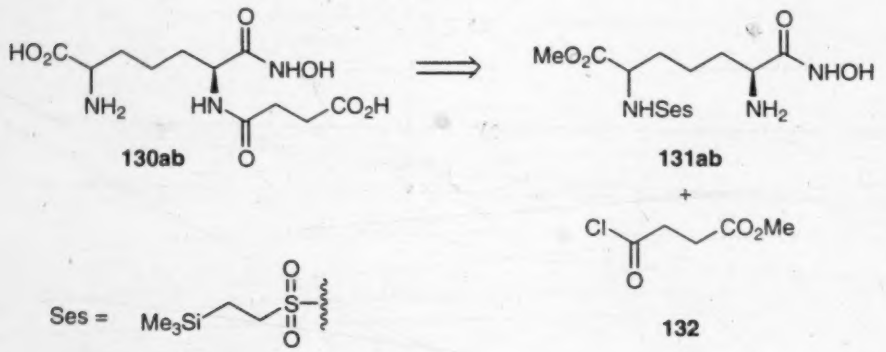
2.3 Synthesis of Succinyl-DAP Hydroxamic Acid 130ab

As hydroxamic acid analogue **94ab** is a substrate for both *meso*-DAP dehydrogenase and DAP epimerase, the possibility of inhibiting the zinc-dependent DAP

desuccinylase with hydroxamic acid analogue **130ab** was investigated. The hydroxamate moiety, mimicking the carboxylate and in proximity to the *N*-succinyl amide bond being hydrolyzed, could coordinate the zinc required for catalytic activity and inhibit DAP desuccinylase.

Initially, it was realized that the synthesis of the hydroxamic acid analogues of succinyl-DAP might be accomplished using the methodology explored previously for the generation of analogue **94ab**. From a retrosynthetic perspective, target **130ab** could be obtained from the condensation of intermediate **131ab** with methyl-4-chloro-4-oxo-butyrate (**132**), as depicted in Scheme 42.

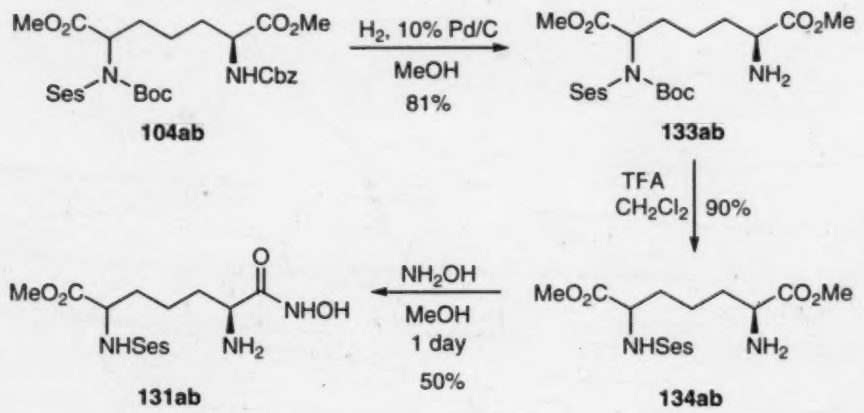
Scheme 42. Retrosynthesis of succinyl hydroxamic acid analogue **130ab**.



Compound **104ab** synthesized previously, as outlined in Scheme 34, is converted to the corresponding amine **133ab** in 81% yield using catalytic hydrogenation (Scheme 43). Subsequent removal of the *tert*-butoxycarbonyl functionality with TFA affords diester **134ab** (90%). Nucleophilic attack at the methyl ester α- to the amino moiety, by

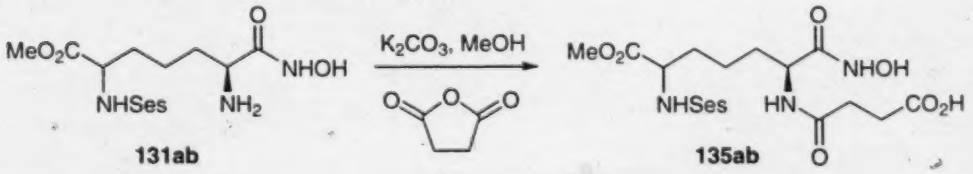
hydroxylamine, results in the formation of hydroxamic acid **131ab** in 50% yield with recovery of starting material (25%).

Scheme 43. Synthesis of hydroxamic acid **131ab** from **104ab**.



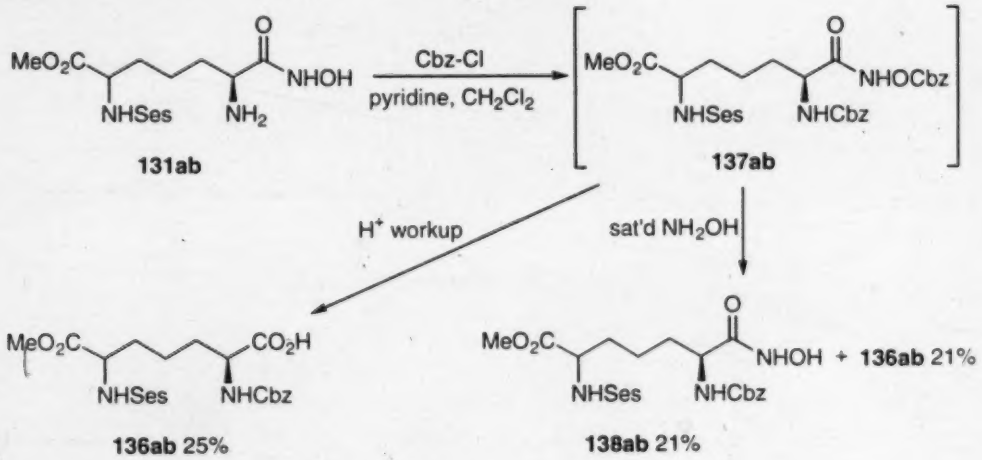
Initial attempts to transform **131ab** to the corresponding succinyl-protected derivative using succinic anhydride results in the formation of a complex mixture of products, including a low yield (< 5%) of the desired compound **135ab** (Scheme 44).

Scheme 44. Reaction of **131ab** with succinic anhydride.



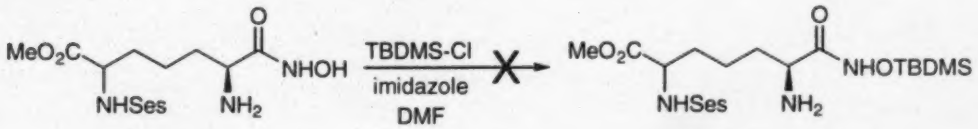
In an effort to find the optimal conditions for reprotection of the amino functionality of **131ab**, benzyl chloroformate was used. Intermediate **131ab** reacts with benzyl chloroformate in the presence of pyridine, followed by acidic treatment to afford compound **136ab** in 25% yield (Scheme 45). Mass spectrometry of the crude reaction mixture suggests that activated hydroxamate **137ab** forms and subsequent exposure to water generates a carboxylic acid functionality. To confirm this hypothesis, the crude reaction mixture is treated with a saturated solution of hydroxylamine in water, giving both the desired hydroxamic acid **138ab** (21%) and carboxylic acid **136ab** (21%).

Scheme 45. Reaction of **131ab** with benzylchloroformate.



As the presence of a free hydroxamate is problematic for acylation of the amino group, several attempts were made to synthesize a protected hydroxamic acid. However, treatment of intermediate **131ab** with *tert*-butyldimethylsilyl chloride and imidazole fails to give ester **139ab** (Scheme 46).

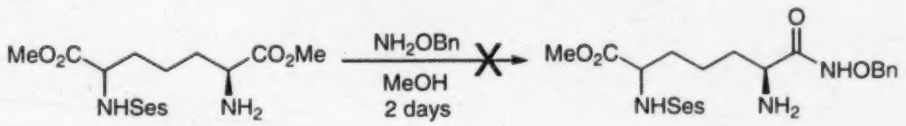
Scheme 46. Attempted reaction of **131ab** with *tert*-butyl-dimethylsilyl chloride.



131ab **139ab**

Furthermore, reaction of diester **134ab** with *O*-benzyl hydroxylamine according to a literature procedure[178] does not produce the expected benzyl derivative **140ab** (Scheme 47), and gives only recovered starting material.

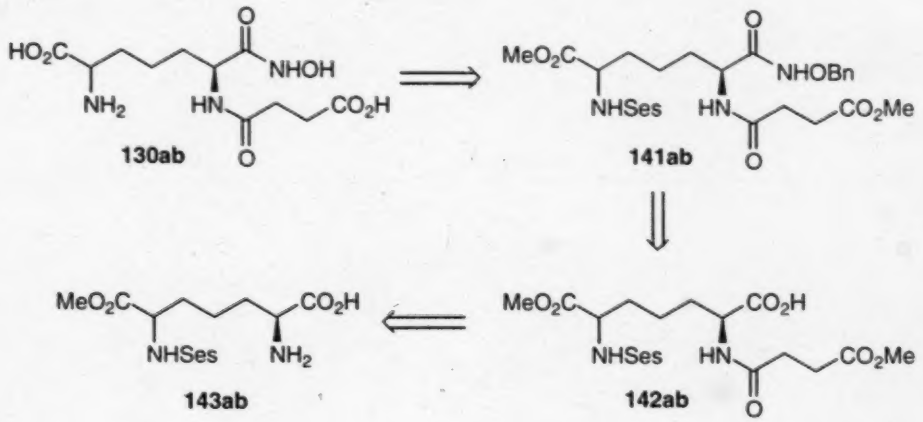
Scheme 47. Attempted reaction of **131ab** with *O*-benzyl hydroxylamine.



134ab **140ab**

The above results indicate that nucleophilic displacement of a methyl ester by a protected hydroxylamine is not a viable approach to obtain hydroxamate **130ab**. Instead, selective removal of one ester functionality to provide **143ab** was envisioned to be necessary to achieve insertion of the hydroxamate moiety at the distal end of the succinyl-DAP analogue (Scheme 48).

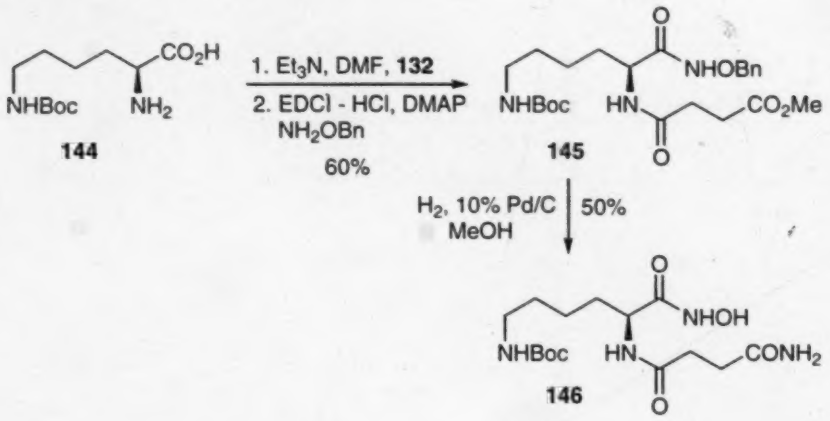
Scheme 48. Retrosynthesis of succinyl hydroxamic acid analogue **130ab**.



Since both succinyl and hydroxamate groups are added to the DAP derivatives in the final steps of the synthesis, lysine derivative **144** was chosen as a model to test the proposed methodology (Scheme 49). Reaction of compound **144** with acid chloride **132** in the presence of triethylamine, followed by coupling with *O*-benzyl hydroxylamine[154] affords intermediate **145** in 60% overall yield. Benzyl hydroxamate **145** is deprotected with catalytic hydrogenation in methanol. Purification by silica gel chromatography with isopropanol:ammonia generates amide derivative **146** in 50% yield.

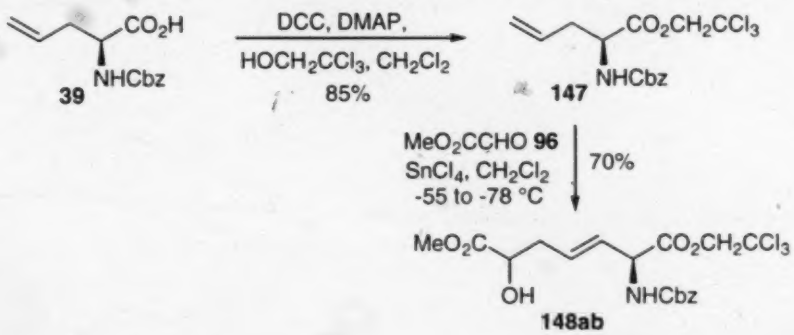
Application of this methodology to the pursuit of DAP analogue **130ab** would require two different carboxyl protecting groups on either side of the DAP skeleton. To achieve this goal, removal of one of the ester protecting groups while maintaining the integrity of other functionalities would be required. The trichloroethyl ester, which can be removed using relatively mild conditions, was thus chosen for our synthetic investigations.

Scheme 49. Synthesis of L-lysine hydroxamic acid derivative **146**.



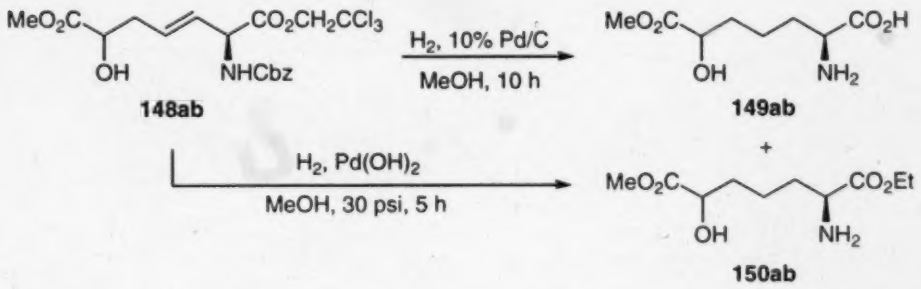
Treatment of *N*-Cbz allylglycine **39** with 2,2,2-trichloroethanol, DCC and a catalytic amount of DMAP using the procedure of González *et. al.*,[179] affords the trichloroethyl ester **147** in 85% yield (Scheme 50). Subsequent condensation of olefin **147** to methyl glyoxylate **96**[159] in the presence of tin (IV) chloride provides alcohol **148ab** (70%) as a 1:1 mixture of two diastereomers that is used for the remainder of the synthesis.

Scheme 50. Ene reaction of 2,2,2-trichloroethyl ester **147** with methyl glyoxylate **96**.



Catalytic hydrogenation of olefin **148ab** using 10% Pd/C fails to give the desired saturated trichloroethyl ester, and instead provides mono-acid **149ab** (12%) and ethyl ester **150ab** (70%) as the major products (Scheme 51). In a second attempt, treatment of **148ab** with 10% $Pd(OH)_2$ in methanol[180] affords solely compound **150ab** in 75% yield. The presence of an ethyl and methyl ester in **150ab** does not allow selective hydrolysis. In an effort to preserve the trichloroethyl ester, an attempt was made to perform the next step, Mitsunobu reaction, with unsaturated derivative **148ab**.

Scheme 51. Catalytic hydrogenation of olefin **148ab** using 10% Pd/C.

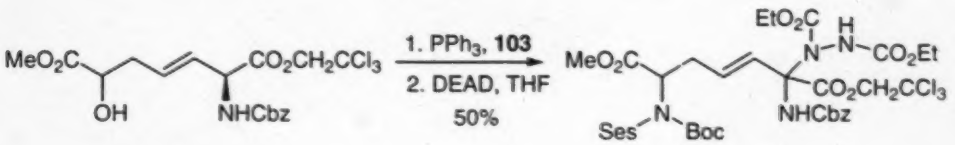


Addition of carbamate **103** and triphenylphosphine to olefin **148ab**, followed by addition of DEAD unexpectedly generates adduct **151a-d** as a mixture of four diastereomers (Scheme 52). The structure of **151a-d** can be confirmed by proton NMR spectroscopy as well as by COSY and HMQC experiments. For instance, the COSY spectrum reveals two olefinic protons at 5.70 ppm and 6.00 ppm that couple to each other and to the neighbouring methylene protons at 2.66-3.06 ppm. Furthermore, the only α-H

at 4.85 ppm couples to this methylene group in the COSY. The two ethyl groups of the diethoxycarbonylhydrazino moiety are also clearly evident in the ^{1}H and ^{13}C spectra.

Addition of diazodicarboxylate species to olefins in an 'ene' type reaction is known in the literature.[181] Initially, it was assumed that the excess DEAD used in the above reaction is responsible for the formation of this adduct. However, when the reaction is performed with equimolar amounts of DEAD and olefin, the same product is obtained.

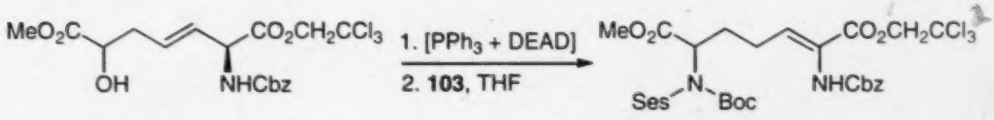
Scheme 52. Mitsunobu reaction of olefin **148** with carbamate **103**.



148ab **151a-d**

In order to investigate the mechanism of this transformation, the Mitsunobu reaction was done with the sequence of reagents altered. Reaction of unsaturated alcohol **148ab** with the preformed PPh_3-DEAD ylide, followed by treatment with carbamate **103** gives α,β-unsaturated intermediate **152ab** (13%), adduct **151a-d** (8%), as well as recovered starting material (16%) (Scheme 53). On the basis of these findings, a mechanism for the formation of the two unsaturated analogues **151a-d** and **152ab** was proposed (Scheme 54 and 55). The DEAD present in the reaction mixture (Scheme 54), may react with the Mitsunobu product **153ab** in an 'ene' type reaction to generate intermediate **154**, which can then rearrange to give **151a-d**.

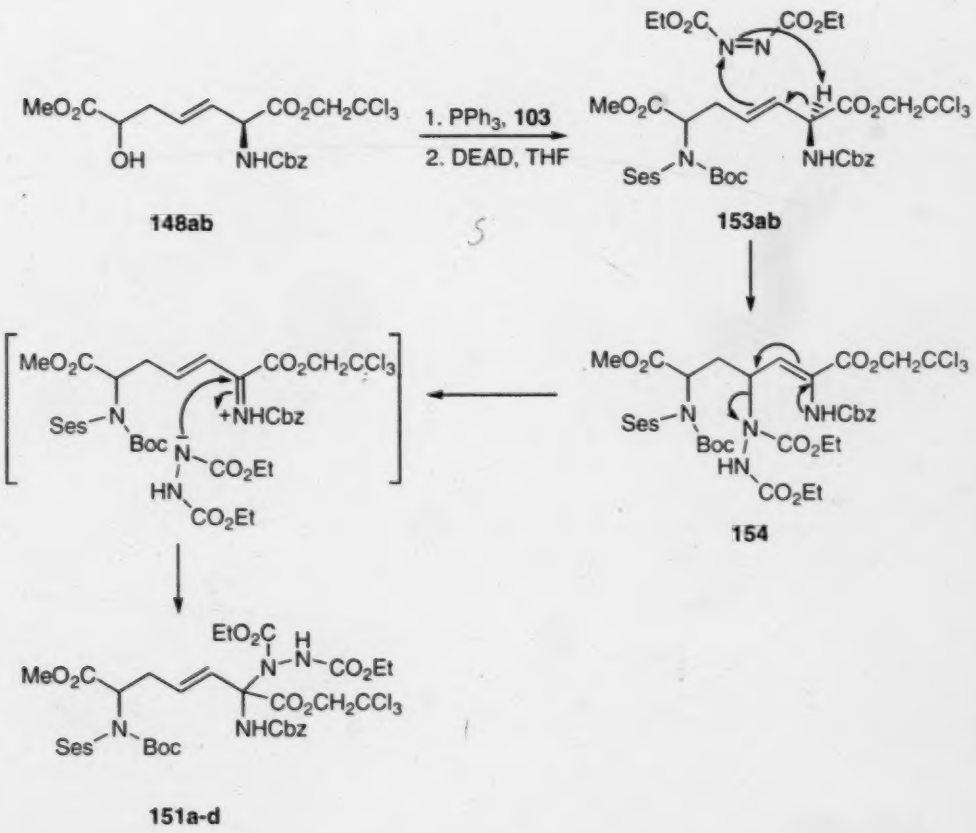
Scheme 53. Mitsunobu reaction of olefin **148** with carbamate **103** and preformed ylide.



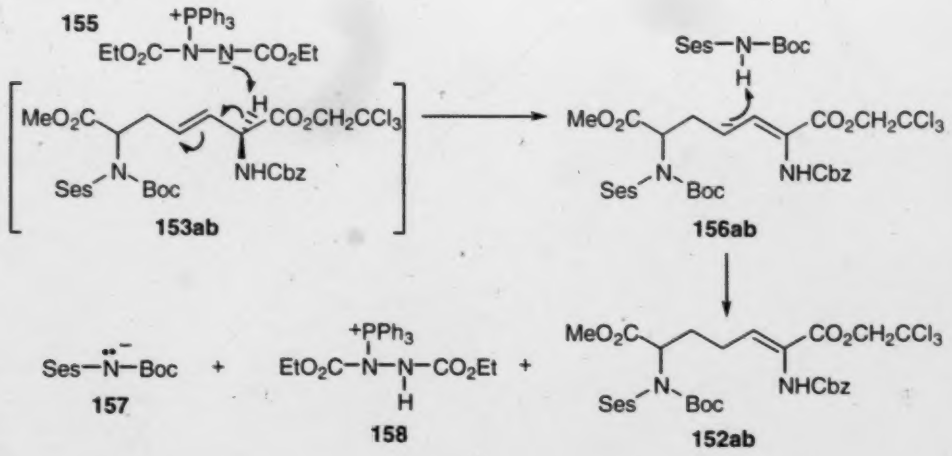
148ab

152ab 13%
+
151a-d 8%

Scheme 54. Proposed mechanism for the formation of **151a-d**.



Scheme 55. Proposed mechanism for the formation of **152ab**.

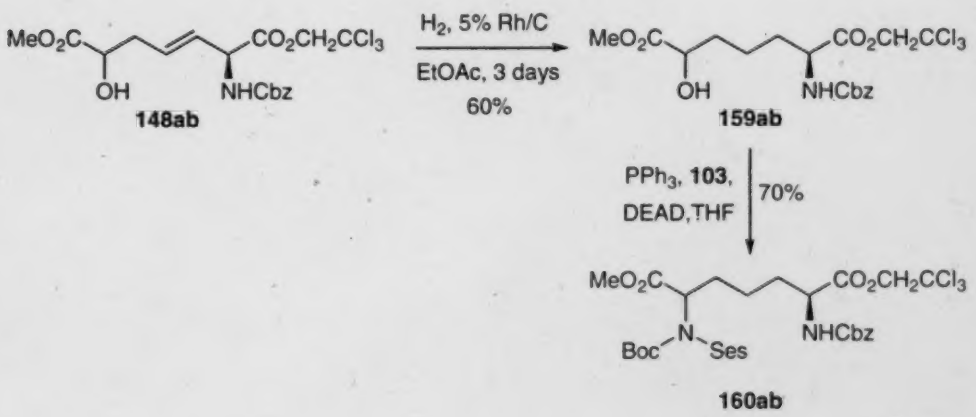


Preforming the ylide **155** by stirring DEAD and PPh_3 for 15 minutes prior to the addition of the olefin and carbamate minimizes the formation of adduct **151a-d**, but leads to deprotonation of the α-hydrogen to afford **152ab** as the major product (Scheme 55). The anion **156ab** formed in this process may be quenched by excess carbamate to give **157** which can react further with the protonated ylide **158**. Although it is not known which reaction is occurring first, it appears as though the 'ene' reaction and/or proton abstraction are competing with the Mitsunobu process.

The above results indicate that performing the Mitsunobu reaction with an unsaturated compound will not lead to the desired product. Thus, different conditions for the removal of the double bond in intermediate **148ab** were investigated. Rhodium metal is known to catalyze the hydrogenation of double bonds without the hydrogenolysis often seen with palladium.[182] Reaction of alcohol **148ab** under an atmosphere of hydrogen with

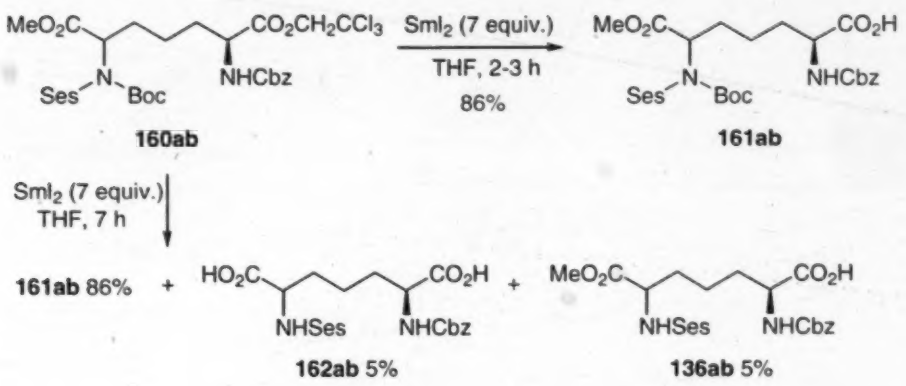
a catalytic amount of 5% Rh/C in ethyl acetate, furnishes saturated compound **159ab** in 60% yield, as depicted in Scheme 56. As expected, Mitsunobu reaction with carbamate **103** affords DAP derivative **160ab**.

Scheme 56. Conversion of olefin **148ab** to amide **160ab**.



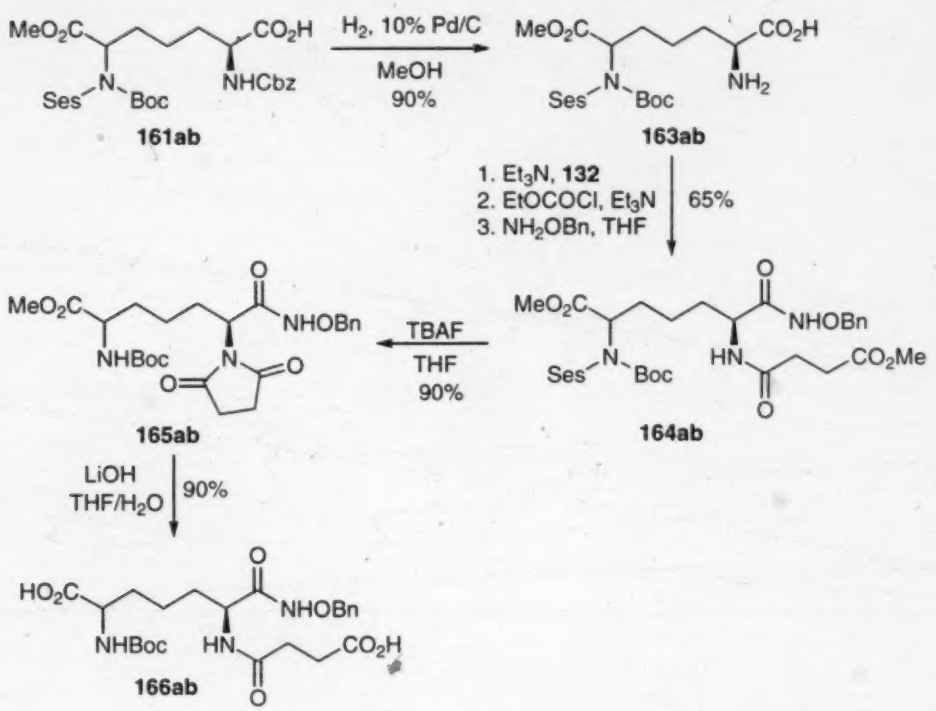
The next step in this synthesis towards **130ab** involves removal of the 2,2,2-trichloroethyl ester protecting group. Treatment of compound **160ab** with samarium (II) iodide in THF[183] for 3 hours gives acid **161ab** in 86% yield (Scheme 57). If this reaction is stirred for 7 hours or longer, Boc deprotection and methyl ester hydrolysis can also occur to give by-products **162ab** (5%) and **136ab** (5%).

Scheme 57. Reaction of 2,2,2-trichloroethyl ester **160ab** with samarium (II) iodide.



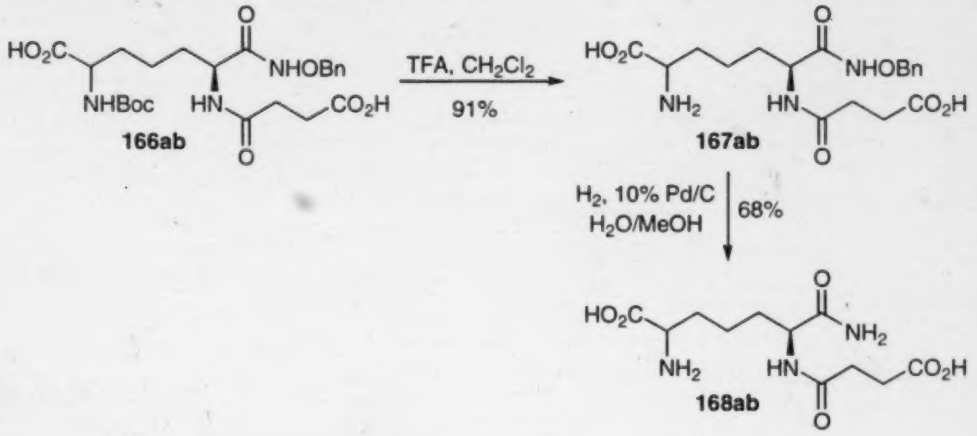
The removal of the Cbz protecting group from **161ab** is realized using catalytic hydrogenation and affords amino acid **163ab**, as shown in Scheme 58. The succinyl group is then installed using acid chloride **132**. Activation of the carboxylic acid by formation of the mixed anhydride, followed by treatment with *O*-benzylhydroxylamine gives benzyl hydroxamate **164ab** in 65% overall yield. Removal of the silyl-protecting group is then achieved by treating intermediate **164ab** with tetrabutylammonium fluoride in THF. The reaction conditions used to remove the Ses group also result in the cyclization of the succinyl moiety to provide succinimide derivative **165ab**. Treatment of **165ab** with three equivalents of lithium hydroxide leads to hydrolysis of the methyl ester, and ring opening of the succinimide ring to generate diacid **166ab** in 90% yield.

Scheme 58. Synthesis of succinyl derivative **166ab** from **161ab**.



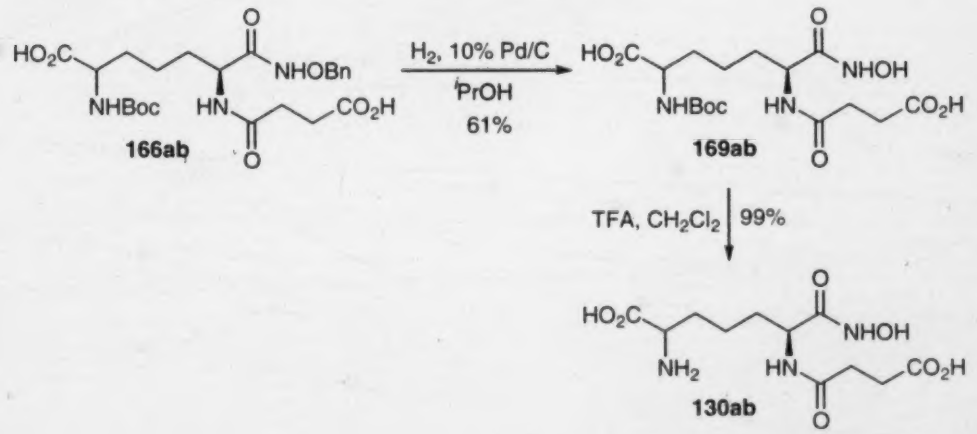
Conversion of compound **166ab** to amine **167ab** (91%) proceeds smoothly with the use of TFA in dichloromethane (Scheme 59). Intermediate **167ab** was purified using an isopropanol/ammonia silica gel column. The ultimate step however, involving hydrogenolytic deprotection of the benzyl hydroxamate unfortunately leads to over-reduction and gives amide **168ab** which fails to spray red to ferric (III) chloride on TLC, a definitive test for hydroxamic acids. The presence of a diammonium salt resulting from purification of **167ab** may facilitate hydrogenolysis of the N-O bond of the hydroxamate.

Scheme 59. Conversion of **166ab** to amide **168ab**.



Hence the order of the deprotection steps was reversed to transform intermediate **166ab**, which was purified by HPLC, to free hydroxamate **169ab** in 61% yield (Scheme 60). The desired DAP analogue **130ab** is finally obtained in 99% yield by removal of the Boc protecting group in **169ab** with TFA.

Scheme 60. Synthesis of hydroxamic acid analogue **130ab** from **166ab**.



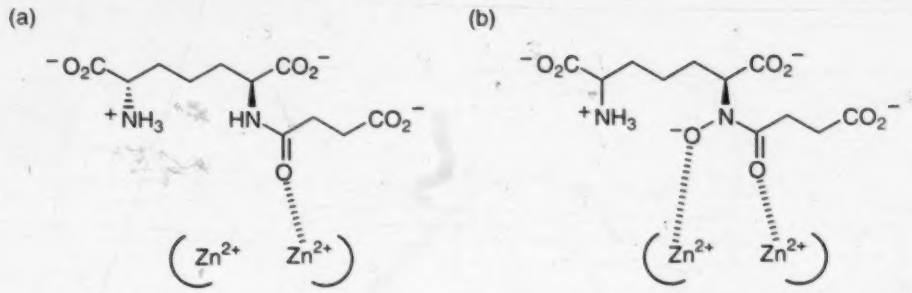
2.4 Inhibition Studies with Succinyl-DAP Hydroxamate 130ab and DAP Desuccinylase

Analogue **130ab** was tested against purified DAP desuccinylase (obtained from Professor J. S. Blanchard's research group) by monitoring a decrease in amide bond absorbance due to succinyl DAP desuccinylation at 220 nm.[75] The substrate used for the assays was a mixture of D,D- and L,L-succinyl DAP, previously synthesized by Dr. William Sherwin in our research group according to the literature procedure.[69]

Enzymatic studies reveal that analogue **130ab** is neither a substrate nor an inhibitor of the enzyme at millimolar concentrations. These results reveal that the hydroxamic acid moiety may be interacting with other amino acid residues of the enzyme, or is simply not in close proximity to the catalytic zinc bound in the active site as initially proposed.

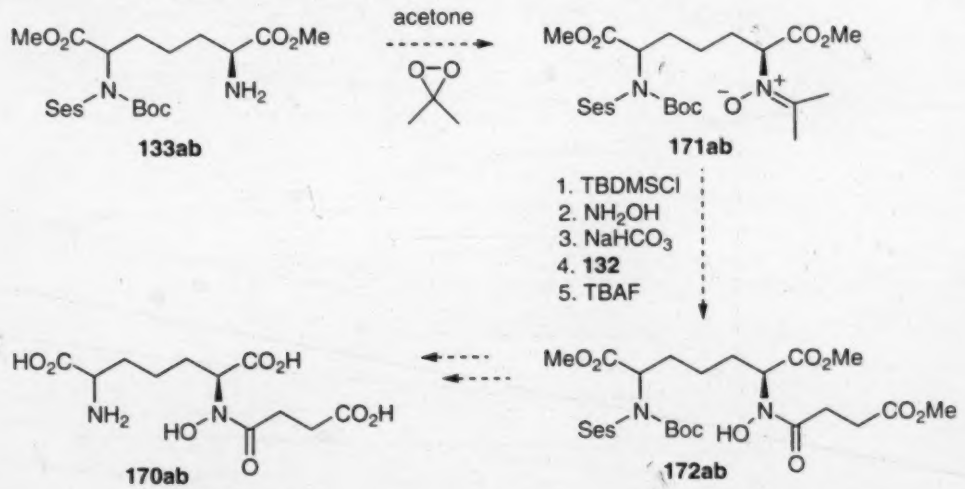
Most of the reported hydroxamate-bearing inhibitors of zinc-containing enzymes are in the form of HN(OH)-COR in which R represents oligopeptides which can be accommodated in the binding pockets of the target enzymes.[184] A possible alternative inhibitor can be envisioned wherein the hydroxamate fuctionality can chelate the active site zinc ion, as depicted in Figure 15.

Figure 15. Schematic representation of binding mode of (a) substrate and (b) hydroxamate-type inhibitor **170**.



A possible synthetic approach for the future synthesis of hydroxamate **170ab** could involve oxidation of previously synthesized intermediate **133ab** with dimethyldioxirane[185] followed by treatment with hydroxylamine and acylation with acid chloride **132** (Scheme 61). Deprotection of the acids and amino group should then provide **170ab**.

Scheme 61. Proposed synthesis for hydroxamic acid derivative **170ab**.



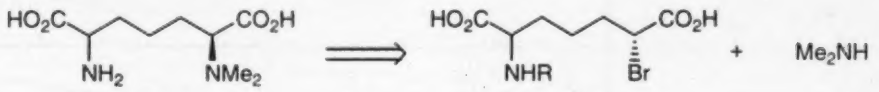
3. Design of DAP Substrates for Peptidoglycan Incorporation

One intriguing question which remains to be addressed is the possible design of DAP derivatives that could act as substrates for one of the key bacterial enzymes and become incorporated into the peptidoglycan chain causing cell wall defects. Cross-linkage of the murein strands is achieved *via* peptide bond formation between the carboxyl group of the terminal amino acid of one peptide moiety and the non-alpha amino group present in the side chain protruding from a neighbouring glycan stand. The majority of cross-links in Gram-negative bacteria is found between the carboxyl group of a terminal D-Ala and the ω-amino group at the D-center of *meso*-DAP (Figures 3 and 4). Thus, if a DAP analogue possessing an *N,N'*-dimethyl moiety at the ω-position is a substrate for one of the enzymes in bacterial lysine biosynthesis, it could become incorporated into the murein and thereby prevent further cross-linkage.

3.1 Synthesis of Dimethylamino DAP Analogue 173ab

The synthesis of DAP derivative **173ab** could potentially be realized by the nucleophilic displacement of bromine in **174ab** by dimethylamine, as outlined Scheme 62.

Scheme 62. Retrosynthesis of dimethlamino DAP analogue **173ab**.



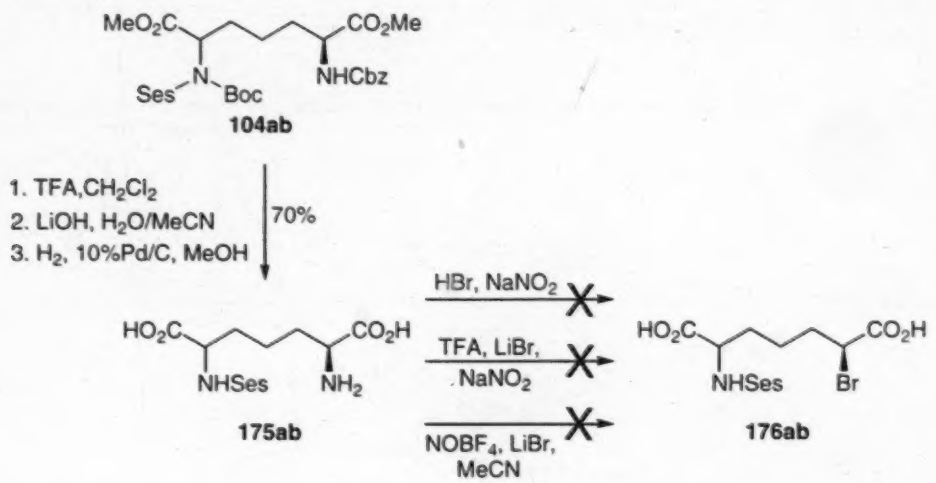
173ab **174ab**

Although a stereocenter with *R* configuration is required at the distal end of the bromo derivative **174ab**, intermediate **104ab** which has an *S* stereocenter is readily available and could be used for model studies to examine this proposed methodology in detail. Diester **104ab** would also help ascertain what functionalities are stable to the proposed reaction conditions.

Removal of the Boc protecting group from **104ab** with TFA, followed by hydrolysis of the methyl esters and deprotection of the Cbz moiety, provides amino acid **175ab** in 70% overall yield (Scheme 63). Several attempts were made to introduce the bromide using diazotization conditions in the presence of a bromine source. In the first attempt, hydrobromic acid and sodium nitrite are used following the procedure of Mori *et. al.*[186] Proton NMR of the crude product reveals that the trimethylsilyl protecting group is cleaved.

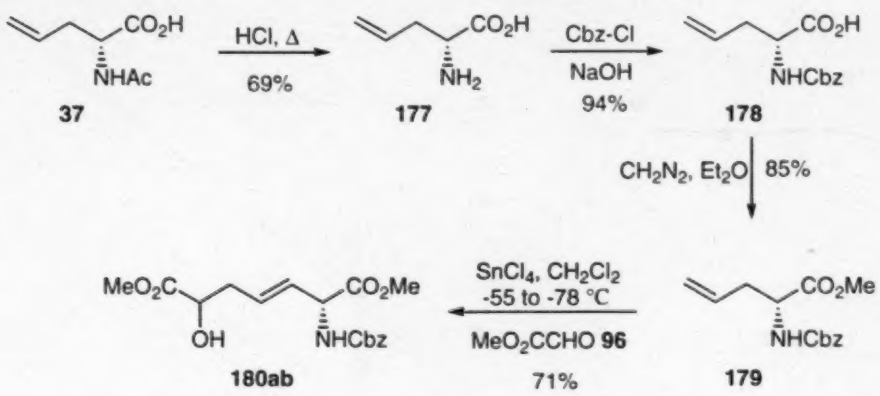
In an effort to preserve the Ses protecting group, **175ab** is reacted with a mixture of trifluoroacetic acid, sodium nitrite and lithium bromide,[187] but unfortunately this does not lead to the desired product **176ab**. Treatment of compound **175ab** with nitrosonium tetrafluoroborate[187] and lithium bromide in acetonitrile also leads to the cleavage of the silyl group and generation of complex mixtures.

Scheme 63. Attempted conversion of **104ab** to bromo derivative **176ab**.



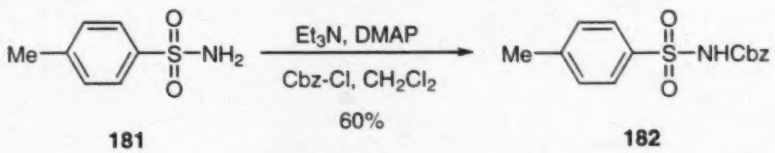
Hence the required DAP skeleton of **174ab** that possesses an *R* stereocenter at the distal end of the molecule, was targeted using the ene and Mitsunobu chemistry, but starting from D-allyl glycine **177**. D-*N*-acetyl-allyl glycine **37**, synthesized previously (Scheme 11), reacts with aqueous HCl under reflux to afford **177**[97] in 69% yield as outlined in Scheme 64. Protection of the amine using benzyl chloroformate in aqueous base gives **178**. Esterification of acid **178** with ethereal diazomethane[170] provides ester **179** in 85% yield. Ene reaction of methyl glyoxylate **96**[159] and **179** in the presence of tin (IV) chloride produces alcohol **180ab** (71%) as a 1:1 mixture of two diastereomers, which is used for subsequent transformations. At this point, a Mitsunobu reaction is required for the construction of the DAP skeleton.

Scheme 64. Synthesis of unsaturated alcohol **180ab** from D-allylglycine **177**.



The choice of nitrogen nucleophile for the Mitsunobu reaction is critical, as a functional group stable to the acidic conditions used for diazotization in the bromination step is needed. The *N*-(sulfonyl)carbamate chosen as the nitrogen nucleophile for the Mitsunobu reaction was sulfonamide **182**[188] which is easily prepared from *p*-toluenesulfonamide (**181**) and benzyl chloroformate (Scheme 65).

Scheme 65. Synthesis of carbamate **182** from *p*-toluenesulfonamide **181**.



In the next step of the synthesis towards **173ab**, olefin **180ab** is transformed to saturated analogue **183ab** (70%) *via* hydrogenation followed by treatment with benzyl

chloroformate (Scheme 66). Alcohol **183ab** reacts with sulfonamide **182** under Mitsunobu conditions to afford **184ab**. Hydrolysis of Mitsunobu product **184ab** using three equivalents of lithium hydroxide in aqueous acetonitrile results in the formation of cyclic product **185** instead the desired acyclic acid. Attempts to selectively hydrolyze the diester were carefully monitored by TLC, and reveal that a third equivalent of base is necessary to completely consume the starting material. The structure of compound **185** can be confirmed by several spectroscopic techniques including high resolution mass spectrometry. The COSY and HMQC data, with the corresponding spectral assignments, are illustrated in Figure 16. One of the key features of the COSY spectrum is the olefinic hydrogen H-4 at 6.10 ppm which couples to the methylene protons H-3ab at 2.20 ppm. This methylene is also coupled to H-2a and H-2b at 1.95 and 2.30 ppm. In addition, the HMQC confirms the presence of the olefinic system, as H-4 couples to C-4 at 123 ppm. A proposed mechanism for the formation of **185** is depicted in Scheme 67.

Scheme 66. Synthesis of cyclic derivative **185** from unsaturated alcohol **180ab**.

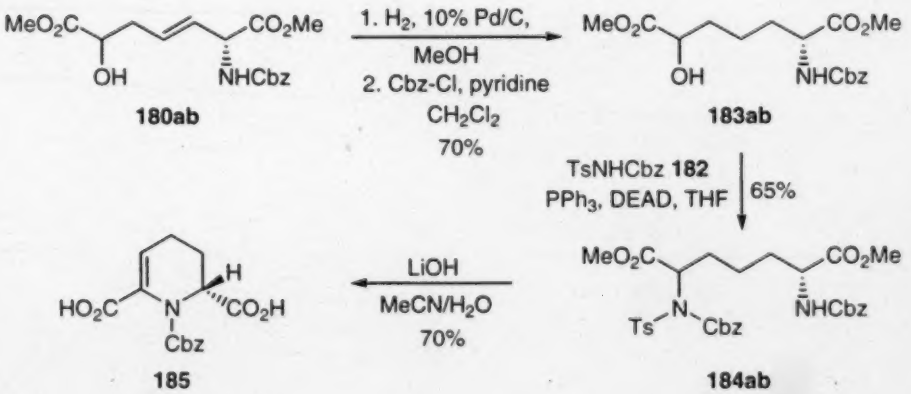
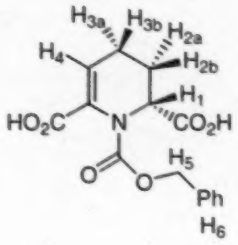
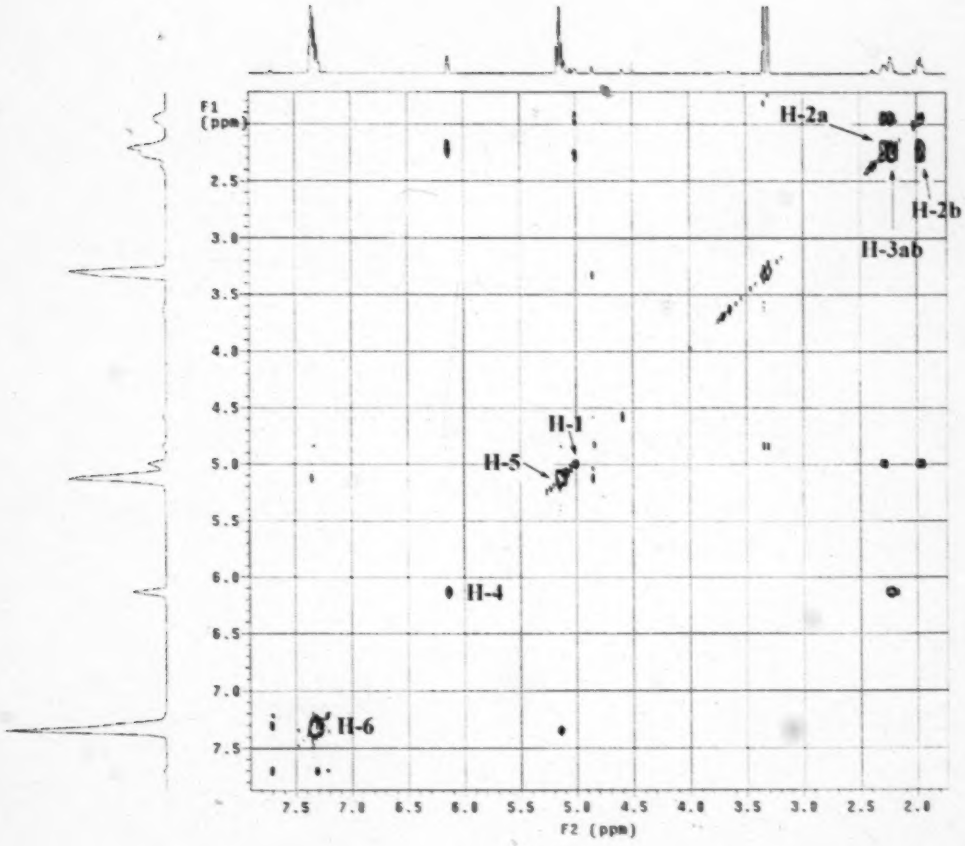


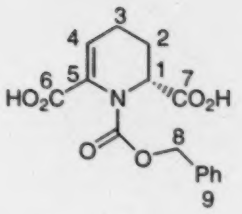
Figure 16. (a) COSY spectrum and (b) ^{13}C-HMQC spectrum for compound **185**.

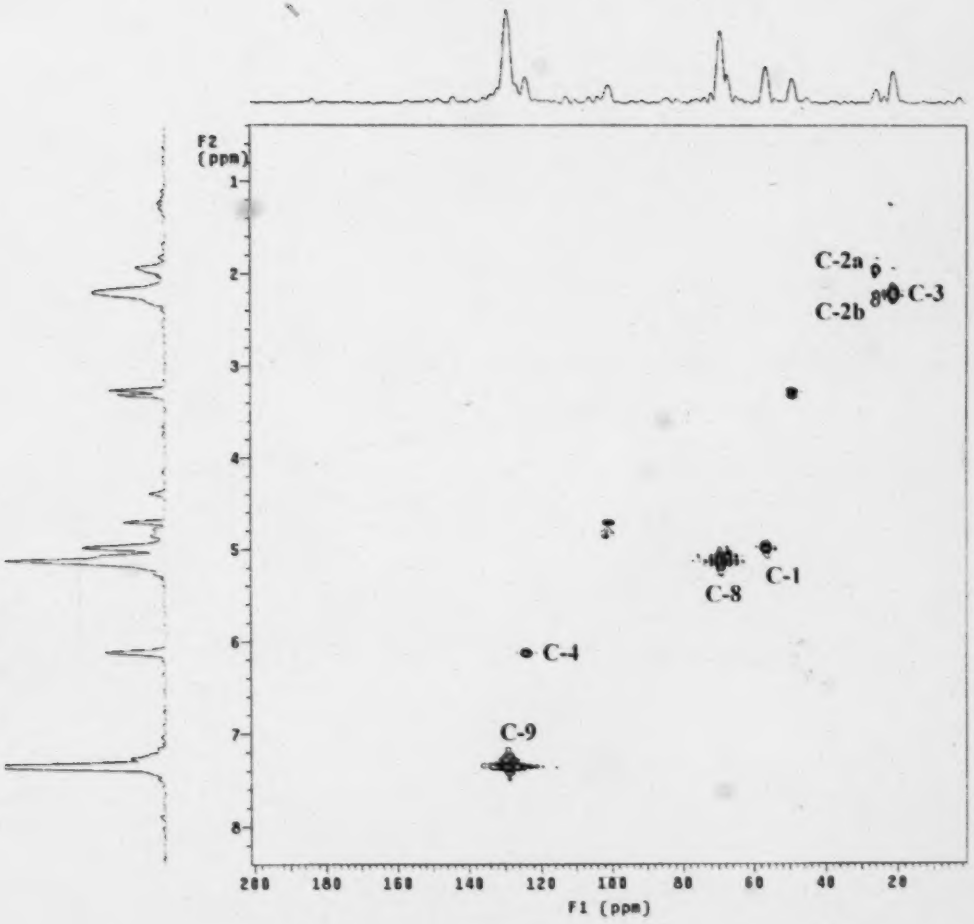
(a) COSY Spectrum in CD_3OD.



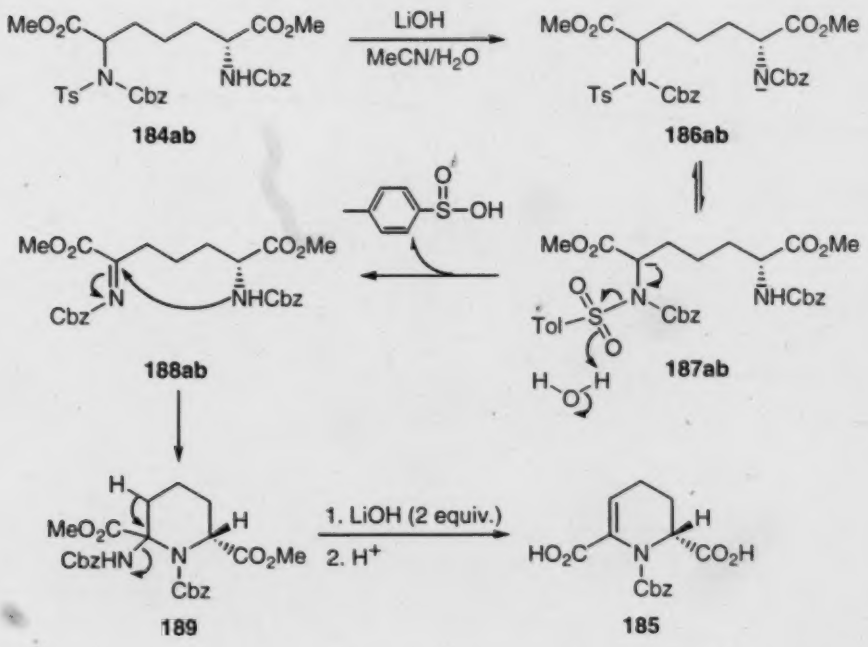


(b) ^{13}C-HMQC in CD_3OD.





Scheme 67. Proposed mechanism for the formation of compound **185**.



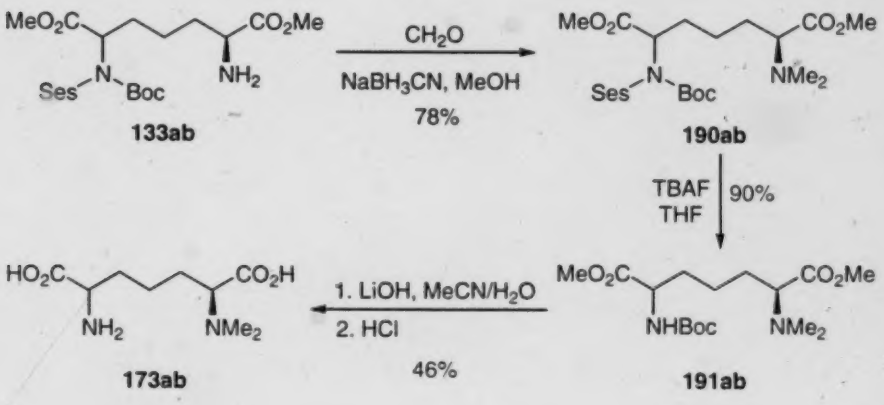
With the failure to successfully deprotect **184ab**, an alternative route was investigated to synthesize target analogue **173ab**. The introduction of methyl groups onto primary amines by reductive alkylation with formaldehyde has proved to be a useful method for the synthesis of dimethylamino derivatives.[189]

Treatment of intermediate **133ab**, previously prepared as outlined in Scheme 43, with six equivalents of formaldehyde followed by sodium cyanoborohydride[190] gives the desired dimethylamine intermediate **190ab** in 78% yield (Scheme 68). Removal of the Ses protecting group using tetrabutylammonium fluoride in THF affords **191ab**, and

subsequent hydrolysis of the methyl esters with lithium hydroxide followed by acidic work-up affords the desired analogue **173ab** in 42% overall yield.

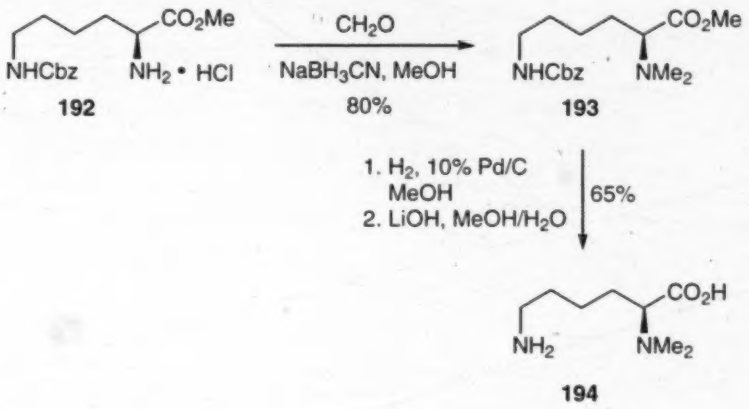
As dimethyl analogue **173ab** would also be tested as a substrate for DAP decarboxylase, a standard sample of the enzymatic product, dimethyl lysine **194**, is needed.

Scheme 68. Synthesis of dimethylamino DAP derivative **173ab** from **133ab**.



Using a modified procedure of Jeffs *et. al.*[190] for the synthesis of dimethylamino compounds, protected lysine **192** is transformed to *N,N´*-dimethyl intermediate **193** in 80% yield (Scheme 69). Hydrogenolytic deprotection of the Cbz protecting group, followed by ester hydrolysis with lithium hydroxide furnishes the *N,N´*-dimethyl L-lysine compound (**194**) in 65% overall yield.

Scheme 69. Synthesis of L-lysine dimethylamino derivative **194**.



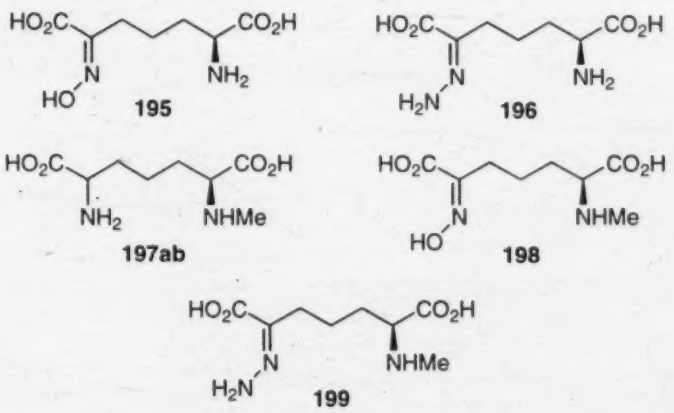
3.2 Enzyme Inhibition Studies with Dimethylamino Analogue 173ab

Inhibition studies with dimethyl analogue **173ab** reveal that it is neither a substrate nor inhibitor for DAP epimerase, *meso*-DAP dehydrogenase or DAP decarboxylase. The presence of the two methyl groups at the distal L-recognition site may impose unfavourable steric interactions with the enzymes, thereby preventing the analogue from binding well in the active site.

Examination of the crystal structure of isoxazole **15** *meso*-DAP dehydrogenase reveals that the distal amino group interacts with Asp90 and Asp120 through hydrogen bonding. Failure of the dimethyl derivative **173ab** to effectively bind in the dehydrogenase active site could also be attributed to the presence of the *N*-alkyl groups which interfere with hydrogen bonding.

As *N*-hydroxy DAP **26** is known to inhibit DAP epimerase, dehydrogenase, decarboxylase and *meso*-DAP adding enzyme, the generation of new DAP analogues such as as oxime **195** and hydrazone **196** may provide new information concerning the proposed mechanisms of these enzymes (Figure 17). Previous work in our group to generate these compounds has been unsuccessful due to cyclization of the distal amine onto the imine moiety. Synthesis of mono-methyl derivative **197ab** may furnish a potential substrate for one of the above enzymes, as the mono-alkylated amine may still participate in sufficient hydrogen bonding interactions. Furthermore, the synthesis of *N*-methyl derivatives **198** and **199** offers the potential for mimicking imine **14** and preventing intramolecular undesired cyclization reactions.

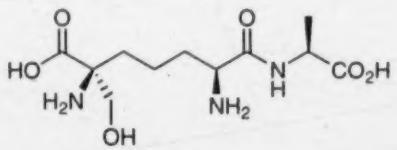
Figure 17. Imine-DAP derivatives as proposed inhibitors.



3.3 Antibacterial Assays

The previously synthesized DAP analogues **31**, **94ab**, **130ab**, and **173ab** as well as lysine derivative **194** were tested as potential antibacterial agents against four strains of bacteria, as outlined in the experimental section. The organisms used for the assay included *E. coli*, *B. sphaericus* IFO 3525, *P. vulgaris* X-19-0, and *C. glutamicum* A5019 PR56. Inhibition of bacterial growth was measured as a halo around the well containing the test compound. Tetracycline and ampicillin were also used as antibacterial controls. The tests revealed that none of these DAP analogues possess antimicrobial activity.

One of the difficulties with DAP inhibitors is their lack of bioavailability, as they are unable to cross the cell membrane. Efficient cellular transport systems are used by microorganisms to recycle its cellular wall components as di- or tri-peptides.[191] In addition, these mechanisms can be used to transport peptidic DAP analogues across the cell membrane.[30] If properly delivered, even modest enzyme inhibitors can be effective antibiotics, as dipeptide derivatives, for example compound **3** which possesses more hydrophobic character. Future work may thus include transformation of previously synthesized DAP intermediates to alanyl dipeptide derivatives.



3

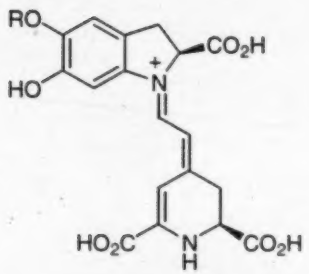
4. Synthetic Studies Toward Betanidin

The betalains are water-soluble, zwitterionic, nitrogenous plant pigments commonly found in species of the order *Centrospermae*.[192] The red-violet betalains belong to a class known as betacyanins whereas the yellow pigments are known as betaxanthins. Examples of plants that contain betalains are cacti, pokeberry and red beet.[193]

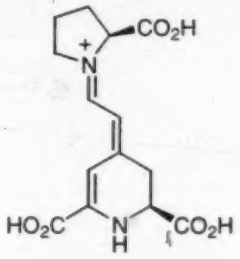
Extensive work on the structures of these alkaloids culminated in 1965 when Dreiding and coworkers proposed a structure for betanin **200**, the red pigment of the beet *Beta vulgaris*[194] (Figure 18). Thereafter, indicaxanthin was isolated from the cactus *Opunita ficus indica Mill*, and shown to have structure **202**.[195] One of the most interesting aspects of the chemistry of these compounds comes from their facile interconversion. Betanin **200**, upon treatment with excess proline in dilute ammonium hydroxide, is converted to **202**.[196] Treatment of indicaxanthin **202** with excess L-cyclodopa **203** gives betanidin **201**. Although it was suspected that betalamic acid **204** was the intermediate in these interconversions, it was not isolated until 1971.[197,198]

The inherent instability and polyfunctional nature of the betalains poses an interesting synthetic challenge. The synthesis of vinylogous amide **82**, which possesses structural similarity to betalamic acid, presented an opportunity to investigate the total synthesis of betanidin. Much effort has been expended in an attempt to synthesize betanidin, particularly by the groups of Büchi and Dreiding.

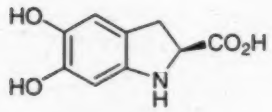
Figure 18. Alkaloid plant pigments and their precursors.



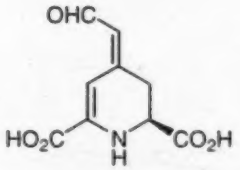
R= Glucose, Betanin **200**
R= H, Betanidin **201**



Indicaxanthin **202**



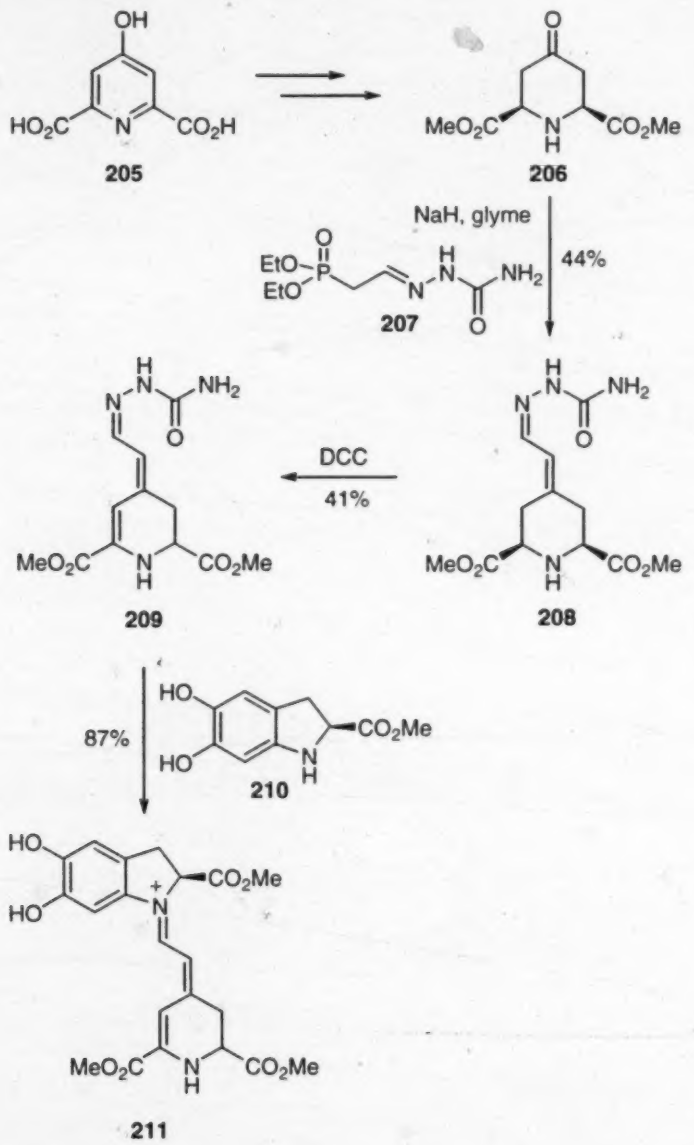
L-Cyclodopa **203**



Betalamic Acid **204**

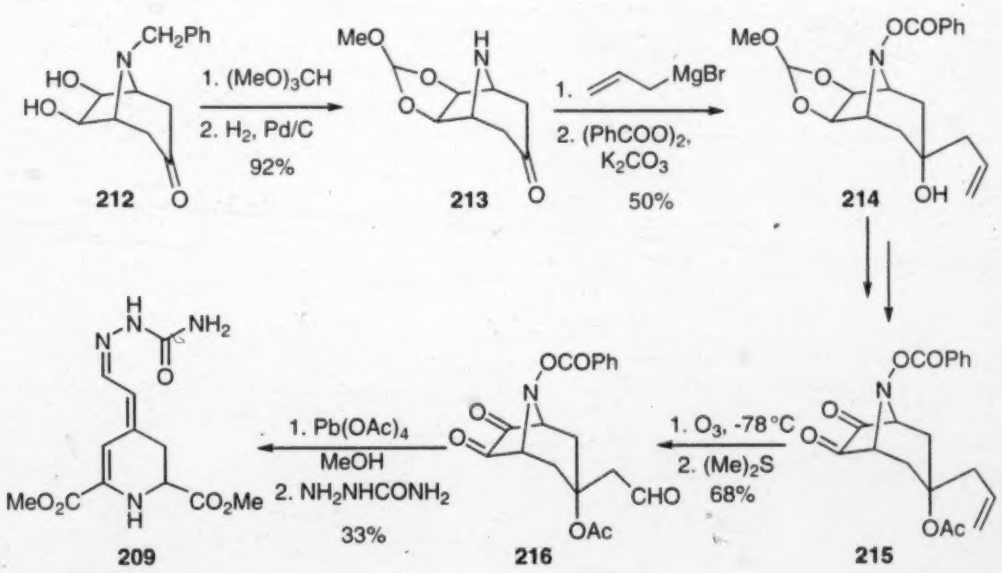
Dreiding reported the first total synthesis of betalamic acid **204** and betanidin **201**.[199] The synthesis starts with chelidamic acid **205** and proceeds in two steps through to the piperidone diester **206** (Scheme 70). Treatment of the diester with a modified Wadsworth-Emmons reagent containing a semicarbazone group gave **208**. This was oxidized with DCC to give the protected betalamic acid derivative **209**. Subsequent transimination with L-cyclodopa methyl ester **210** afforded a diastereomeric mixture of the methyl ester of betanidin **211**. Although a more detailed investigation into the synthesis of betanidin based on the above methodology was later reported[200] Dreiding and coworkers were unable to generate betanidin as a single stereoisomer.

Scheme 70. Dreiding synthesis of betanidin methyl ester **211**.



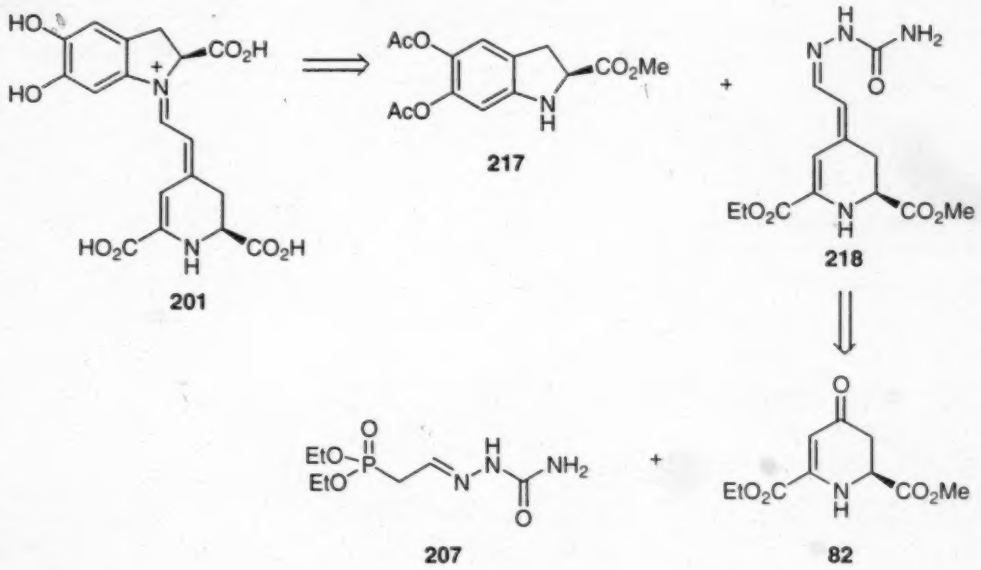
The synthesis of betalamic acid **204**[201] and betalamic acid dimethyl ester semicarbazone **209**[202] have also been reported by Büchi and coworkers. In the synthesis developed for **209**, *N*-benzyl norteloidinone **212** reacts with methyl orthoformate followed by catalytic hydrogenation to give intermediate **213** (Scheme 71). Treatment with allylmagnesium bromide, and subsequent protection of the amine as the benzoylated hydroxylamine affords **214**, which is subsequently transformed in several steps to aldehyde **216**. Conversion of this intermediate to betalamate derivative **209** is finally achieved with lead tetraacetate, followed by semicarbazide. Unfortunately, the Büchi synthesis is also not stereospecific, giving a mixture of diastereomers of dimethyl betalamate semicarbazone **209**.

Scheme 71. Büchi synthesis of betalamate semicarbazone **209**.



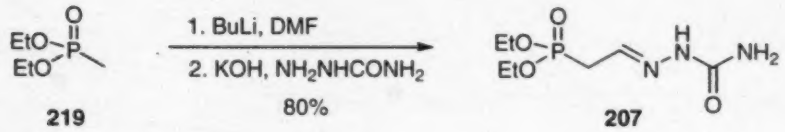
With cyclic vinylogous amide **82** available, an opportunity arose to synthesize betanidin stereospecifically, a goal that had not been accomplished previously. From a retrosynthetic viewpoint, a synthesis similar to the one developed by Dreiding and coworkers was envisioned as outlined in Scheme 72.

Scheme 72. Retrosynthesis of betanidin **201**.



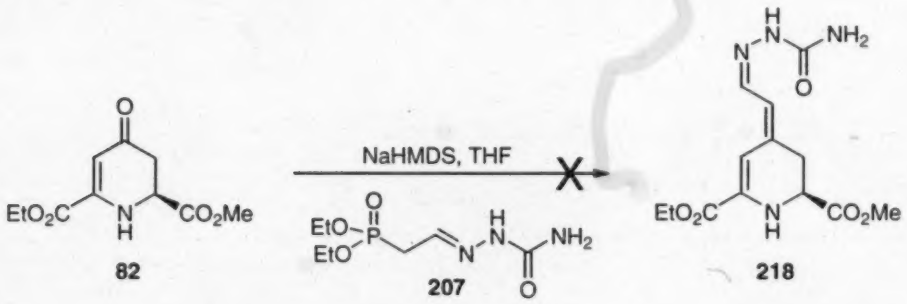
The first step of the total synthesis of betanidin involves the formation of the modified Wadsworth-Emmons reagent using a literature procedure.[203] Treatment of diethylmethane phosphonate **219** with a solution of *n*-butyllithium in hexanes followed by DMF affords the crude (diethoxyphosphinyl) acetaldehyde that is subsequently reacted with semicarbazide to give **207** in 80% yield (Scheme 73).

Scheme 73. Synthesis of modified Wadsworth-Emmons reagent **207**.



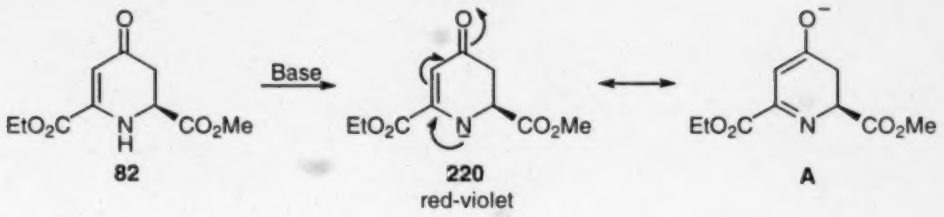
Initial attempts to react vinylogous amide **82** with the ylide generated from the addition of sodium hexamethyldisilazane to Wittig reagent **207**, fail to give betalamate derivative **218** and result in the recovery of starting material (Scheme 74).

Scheme 74. Attempted synthesis of betalamate semicarbazone **218**.



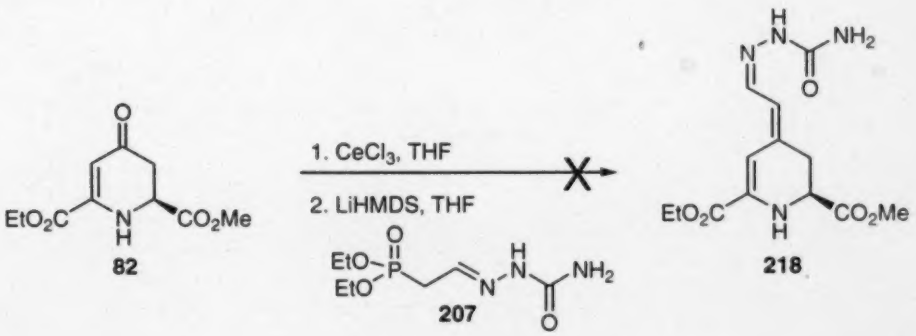
It is interesting to note that the addition of a basic solution of **207** in THF to compound **82** produces a strong red-violet colour. This colour may be attributed to the formation of anion **220**, whose resonance structure is a conjugated enolate **A** (Scheme 75). By virtue of this electron delocalization, the ketone functionality is deactivated for the Wittig reaction.

Scheme 75. Electron delocalization of anion **220**.



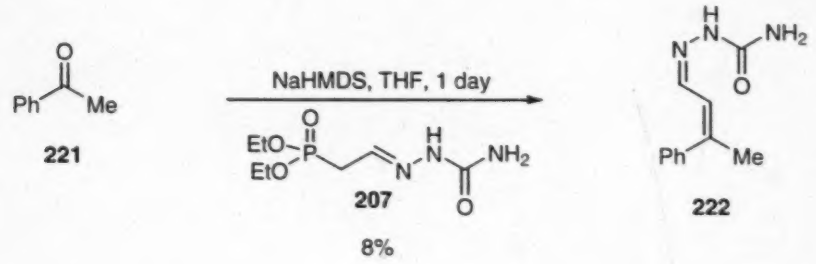
In an effort to hinder amide deprotonation, the above reaction was performed in the presence of anhydrous cerium chloride. It is known that organocerium (III) reagents generated from the reaction of organolithiums with cerium (III) halides undergo efficient carbonyl addition, even if the substrates are susceptible to enolization.[204] Furthermore, cerium chloride has a strong oxophilicity and is capable of activating carbonyl components by coordination.[205] Addition of cerium chloride may also weaken the strong basicity of the Wittig reagent. Unfortunately, the use of cerium chloride offers no improvement over previously obtained results (Scheme 76).

Scheme 76. Attempted synthesis of **218** in the presence of cerium chloride.



In light of these disappointing results, a Wittig reaction with commercially available starting material was done in an attempt to examine the reactivity of the Wadsworth-Emmons reagent **207**. Acetophenone **221** reacts with a mixture of sodium hexamethyldisilazane and compound **207** to give the desired olefin **222**[206] in only 8% yield with 70% recovery of the starting material (Scheme 77).

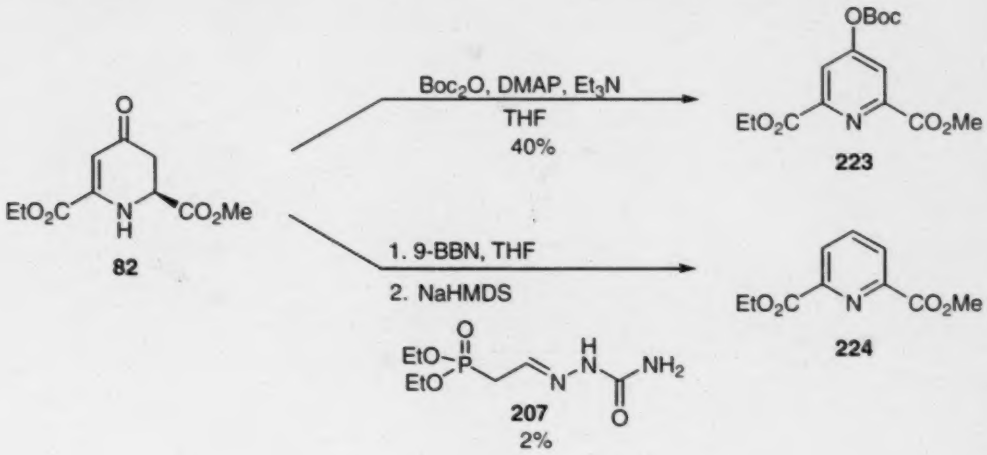
Scheme 77. Synthesis of semicarbazone **222** with Wadsworth-Emmons reagent **207**.



Despite the low reactivity of this stabilized ylide, it was decided to continue the synthesis of betanidin and focus efforts on protecting the nitrogen of cyclic amide **82** so as to prevent deprotonation at this center. Compound **82** reacts with di-*tert*-butyl pyrocarbonate, in the presence of triethylamine and a catalytic amount of DMAP to afford the pyridine derivative **223** (40%) and recovered starting material (20%), as shown in Scheme 78. Acylation apparently generates a sensitive dihydropyridine system, which is readily oxidized by air. Treatment of **82** with 9-BBN, followed by a basic solution of **207** affords recovered starting material (50%) and a trace amount of pyridine derivative **224** (2%) as confirmed by proton NMR and mass spectrometry. Presumably this arises by carbonyl reduction followed by elimination and oxidation. It was thus decided to protect

the nitrogen of the vinylogous amide in the absence of base in order to circumvent the formation of these pyridine derivatives.

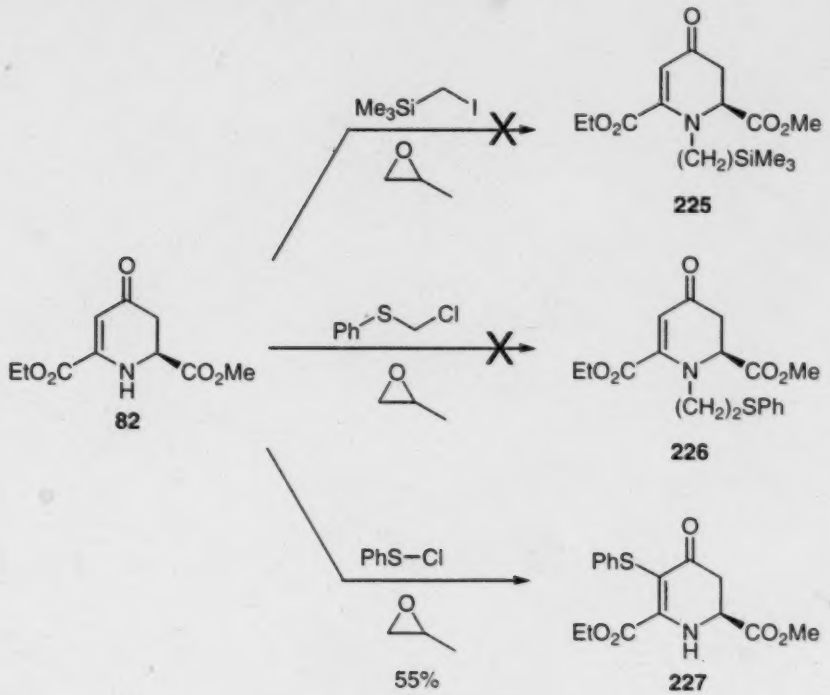
Scheme 78. Formation of pyridine derivatives from vinylogous amide **82**.



Attempts to react amide **82** with (iodomethyl)trimethylsilane or (chloromethyl)sulfenyl benzene in the presence of propylene oxide fails to give the desired *N*-alkylated derivatives **225** and **226** (Scheme 79) and gives only recovered starting material. Treatment of **82** with more reactive phenylsulfenyl chloride results in the formation of a *C*-alkylated intermediate **227** in 55% yield. Apparently the combination of steric hindrance at the nitrogen and delocalization of the nitrogen lone pair prevents *N*-protection. Due to the lack of success at protecting the nitrogen of amide **82**, it seemed that the target *N*-protected cyclic vinylogous amide could be made by cyclization of the corresponding acyclic precursor (see Scheme 26) with the *N*-protecting

group already in place. Amines protected by the Cbz functionality are known to undergo cyclization reactions.[207] Hence, the next target was an open-chain vinylogous amide with the distal amine possessing a Cbz group.

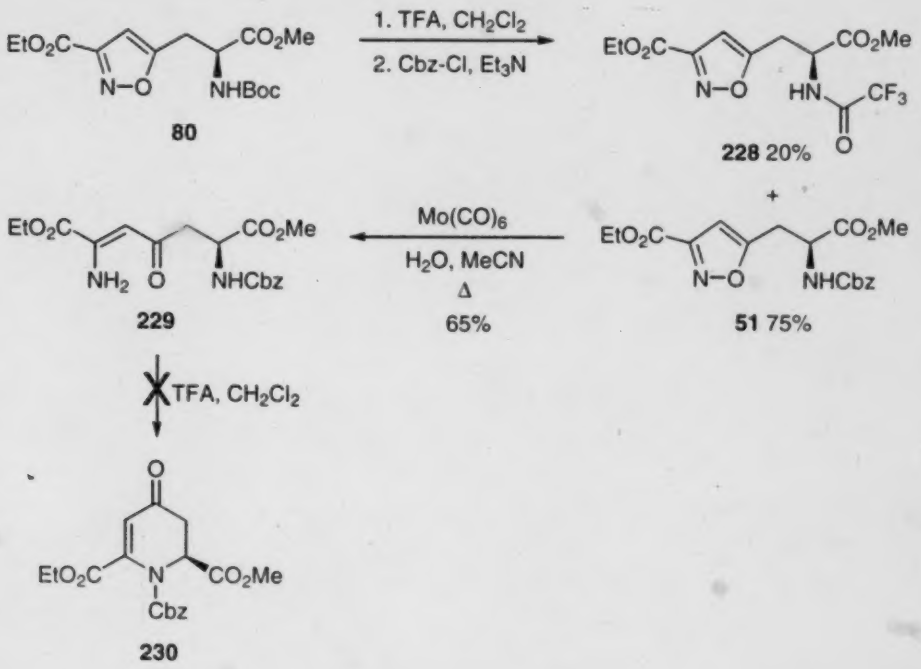
Scheme 79. Reaction of vinylogous amide **82** with reactive organohalides.



Reaction of **80** with TFA followed by treatment with benzyl chloroformate affords the desired intermediate **51** (75%) in addition to a small amount of trifluoroacetyl-derivative **228** (20%) (Scheme 80). Reductive ring-opening of isoxazole **51** with molybdenum hexacarbonyl generates vinylogous amide **229** in 65% yield.

Unfortunately, reaction of intermediate **229** with TFA fails to give the desired cyclic vinylogous amide **230** and affords only recovered starting material.

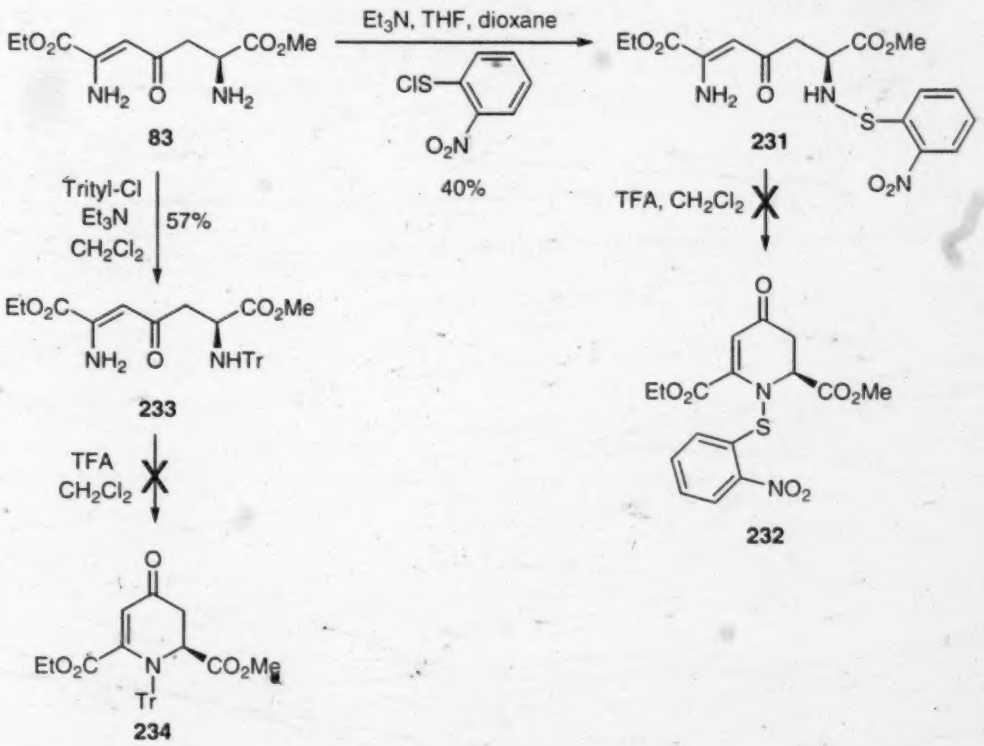
Scheme 80. Synthesis of vinylogous amide **229**.



Since **229** is apparently not nucleophilic enough, two other vinylogous derivatives lacking an amide functionality at the distal end were synthesized. Vinylogous amide **83** is treated with (*o*-nitrophenyl)sulfenyl chloride in the presence of triethylamine to give intermediate **231**, as outlined in Scheme 81. Acid catalyzed cyclization of this vinylogous amide with TFA fails to give cyclic derivative **232**, and affords only the fully deprotected

starting material **83**. Attempts to form a protected cyclized derivative using trityl derivative **233** were also unsuccessful.

Scheme 81. Attempted cyclization of vinylogous amide derivatives **231** and **233**.



The challenging aspect of this synthesis is to prepare betalamate derivative **218** without compromising the integrity of the L-stereocenter of the molecule. Dreiding and Büchi both found that the betanidin **201** and betalamic acid **204** are sensitive to oxidation and easily afford pyridines. Therefore, both groups utilized synthetic strategies in which

unwanted aromatizations could be avoided. Furthermore, Dreiding and coworkers introduced the double bond into cyclic amide **208** after the Wittig reaction had taken place, as outlined in Scheme 70, perhaps due to difficulties caused by electron delocalization.

Although attempts to synthesize a cyclic derivative capable of undergoing the necessary Wittig reaction were unsuccessful, new synthetic investigations involving protection of the double bond present in intermediate **82** could prevent the delocalization of the electrons in the ring, thus precluding the formation of *O*-acylated or *C*-alkylated derivatives.

SUMMARY AND FUTURE WORK

Several DAP derivatives have been synthesized and tested against key bacterial enzymes involved in lysine biosynthesis (Figure 19) with the aim to gain additional knowledge concerning enzyme mechanism and potential antimicrobial design. Isoxazole **31** was synthesized *via* the oxidation of isoxazoline derivatives using active γ-manganese dioxide. An improved synthesis of isoxazole **31** was also developed using a 1,3-dipolar cycloaddition reaction between ethyl chlorooximidoacetate **46** and protected propargyl glycine derivative **79**. Reductive ring opening of an isoxazole derivative **80** using molybdenum hexacarbonyl furnished the two cyclic vinylogous amides **84** and **85** in one step. This ring cleavage offers a method to prepare other vinylogous amide DAP derivatives as possible inhibitors of bacterial enzymes.

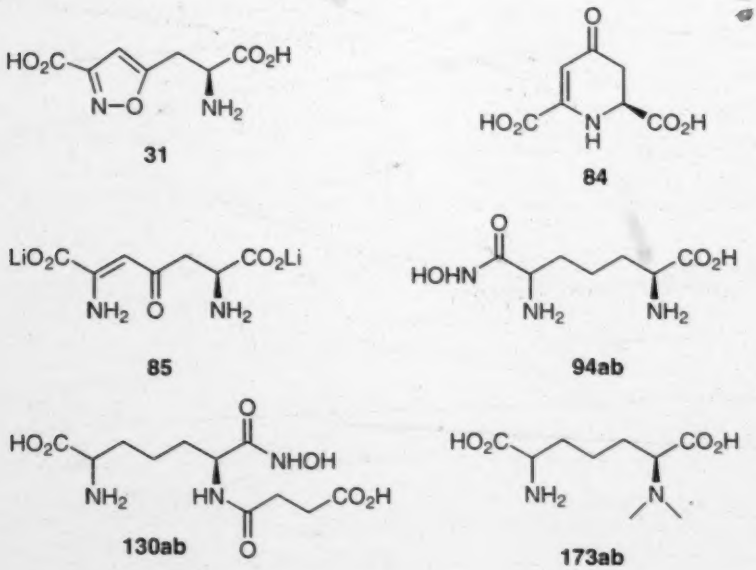
Lack of potent inhibition against *meso*-DAP dehydrogenase or DAP epimerase observed for aromatic DAP analogue **31** and vinylogous amides **84** and **85** suggest that a flexible substrate-analogue may be required in order to effectively inhibit these enzymes. This idea is further confirmed by the fact that olefinic DAP analogues **87** and **88** are potent inhibitors of *meso*-DAP dehydrogenase.[142] Interestingly, cyclic vinylogous amide **84** which shows good structural similarity to L-THDP **7** is a strong competitive inhibitor (K_i 32 μM) of L-DHDP reductase, comparable to pyridine derivative **13**, the most potent inhibitor of the enzyme reported thus far.

The methodology developed for the synthesis of hydroxamic acid **94ab** is valuable for selective incorporation of new carboxylic acid mimics at the distal non-

reacting or α-reacting site of the DAP molecule and could thus be extended to other amino acid derivatives and potential nucleophiles.

Hydroxamic acid **94ab** was shown to be a substrate for *meso*-DAP dehydrogenase with K_m = 1.39 mM ± 0.17 mM and V_{max} = 0.026 mM/min ± 0.002 mM/min. In addition, this compound was shown to be a weak substrate for DAP epimerase, acquiring approximately 10-15% ^2H incorporation after incubation with the enzyme in deuterated buffer. Further, hydroxamate **94ab** was tested against *meso*-DAP adding enzyme, but shows only 82% inhibition at 5 mM. Inhibition studies against DAP decarboxylase reveal that hydroxamate **94ab** is the only analogue obtained from this work to inhibit the enzyme (IC_{50} 0.35 mM), suggesting that decarboxylase has highly specific substrate requirements.

Figure 19. DAP inhibitors used to probe bacterial enzymes.



Succinyl-DAP hydroxamate **130ab** was synthesized from the appropriate DAP intermediate possessing two different ester functionalities. The ease of removal of the trichloroethyl ester moiety from **160ab**, used for the selective insertion of the hydroxamic acid, offers a new method for preparing other DAP analogues, using ene and Mitsunobu chemistry. Inhibition studies reveal that hydroxamate **130ab** is not an inhibitor of the zinc-dependent DAP desuccinylase. It may be that the hydroxamate is simply not capable of binding the zinc needed for catalytic activity.

Although *N,N'*-dimethyl analogue **173ab** displays no inhibition against *meso*-DAP dehydrogenase, DAP epimerase or DAP decarboxylase, preparation of this derivative provides a route to the formation of new imine derivatives as potential inhibitors of these enzymes.

Work is currently in progress to synthesize new DAP compounds based on the knowledge gained from this research. Further functionalization or modification of amide **84** may provide even more potent inhibitors of L-DHDP reductase. In addition, synthesis of hydroxamate **169** could lead to a novel metal chelator that is capable of inhibiting DAP desuccinylase. Synthesis of mono-methyl derivatives **198** and **199** is also being investigated as new inhibitors of *meso*-DAP dehydrogenase and DAP epimerase.

Structural similarity of vinylogous amide **84** to the alkaloid betanidin **201** prompted studies toward the total synthesis of this natural product. Attempts to synthesize betalamate derivative **218** were unfortunately unsuccessful due to the susceptibility of **84** to form pyridine derivatives under the basic conditions of the Wittig reaction which is a key step in the synthesis of betanidin. Modification of vinylogous amide **84** may offer an efficient stereospecific synthesis of the natural product, betanidin.

EXPERIMENTAL PROCEDURES

1. General Procedures

All reagents and solvents utilized were of ACS grade or finer and were used without further purification unless otherwise mentioned. All processes involving air or moisture sensitive reactants and/or requiring anhydrous conditions were performed under a positive pressure of pre-purified argon using oven-dried glassware. Solvents for anhydrous reactions were dried according to Perrin *et al.*[208] Tetrahydrofuran (THF), diethyl ether (Et_2O), and 1,2-dimethoxyethane (DME) were freshly distilled over sodium and benzophenone under an argon atmosphere whereas benzene and toluene were freshly distilled over sodium. Dichloromethane, carbon tetrachloride, acetonitrile, triethylamine, pyridine, *N,N*-diisopropylamine were distilled over calcium hydride. Methanol was distilled over magnesium and iodide. The removal of solvent *in vacuo* refers to evaporation under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (< 0.1 mmHg) to a constant sample weight. Deionized water was obtained from a Milli-Q reagent water system (Millipore Co., Milford, MA). Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, HCl, NaOH, and KOH refer to aqueous solutions. Brine refers to a saturated aqueous solution of NaCl. Diazomethane (CH_2N_2) was prepared using Diazald™ according to the literature procedure.[209] Solutions of *n*-butyllithium were periodically titrated against methanol/phenanthroline.[210]

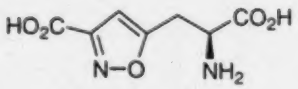
All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using Merck glass-backed plates precoated with normal

silica gel (Merck 60 F254) or reverse-phase gel (Merck RP-8 or RP-18 F254S). One or more of the following methods were used for visualization: UV fluorescence, iodine staining, phosphomolybdic acid/ceric sulfate/sulfuric acid/water (10 g:1.25 g: 12 mL:238 mL) spray for general hydrocarbons, ninhydrin/*n*-BuOH/AcOH (0.32 g:100 mL:3 mL) spray for amino acids and amines, ferric chloride/ 2 M HCl (0.5 g:30 mL) for hydroxamic acids.[211] Flash chromatography was performed according to the Still procedure,[212] using silica gel 60 (Silicycle, 230-400 mesh). The cation exchange resins used for purification of amino acids were BioRad AG 50, 50W (H^+ form, 50-100 mesh). Microcrystalline cellulose for cellulose chromatography was purchased from Merck. HPLC separations were performed on either a Rainin Dynamax instrument equipped with a variable wavelength model UV-1 detector, a solvent delivery system model SD-200, and a Rheodyne injector or the Beckman System equipped with a 166 variable wavelength UV detector and an Altex 210A injector. HPLC separations were monitored at a wavelength of 219 nm. The columns used were Waters C_{18} Resolve 10 µm, Bondpak C_8 or Bondpak C_{18}. Sample solutions were filtered through a 2 µm filter before injection. HPLC grade acetonitrile (190 nm UV cutoff) were obtained from Fisher (Fair Lawn, NJ). All HPLC solvents were filtered daily with a Millipore vacuum filtration system before use.

Ultraviolet spectroscopy was performed on either a GBC Cintra 40 UV spectrometer or a Hewlett Packard 8452A diode array spectrometer. Protein concentrations were determined using bovine serum albumin as a standard with a BioRad Bradford protein assay kit following manufacturer's instructions. Melting points reported have not been corrected and were determined on a Thomas-Hoover or a Büchi oil immersion apparatus using open capillary tubes. Optical rotations were measured on a

Perkin Elmer 241 polarimeter with a microcell (10.00 cm, 0.9 mL) at ambient temperature and are reported in units of 10^{-1} deg cm^2 g^{-1}. All specific rotations reported were referenced against air and were measured at the sodium D line. Infrared (IR) spectra were obtained on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Cast refers to the evaporation of a solution on a NaCl plate, nujol refers to paste made from sample and nujol oil, and (μscope) refers to the use of a microscope to obtain an IR from a crystalline sample. Mass spectra (MS) were obtained using one of the following Kratos AEI instruments: model MS-50 high resolution (HR) mass spectrometer (electron impact ionization (EI)), model MS-9 (fast atom bombardment (FAB), argon), model MS-12 low resolution spectrometer (chemical ionization (CI), NH_3), and Micromass ZabSpec Hybrid Sector-TOF instrument (electrospray ionization (ES)). Cleland matrix was used for FAB experiments and refers to 5:1 mixture of dithiothreitol and dithioerthritol. Microanalyses were determined on a Perkin Elmer 240 or a Carlo Erba 1180 elemental analyzer. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker AM-300 and Inova Varian 300, 500, and 600 MHz instruments. ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in Hertz (Hz), and assignment. For multiplets with large ranges (≥ 0.05 ppm), the range is provided. All literature compounds had IR, ^{1}H NMR and mass spectra consistent with the reported data.

2. Experimental Data for Compounds

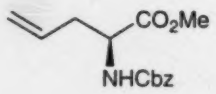


5-((2*S*)-Amino-2-carboxy-ethyl)isoxazole-3-carboxylic acid (31).

Procedure A: To a solution of isoxazole **52** (91 mg, 0.27 mmol) in MeCN (5 mL) under argon was added chlorotrimethylsilane (0.14 mL, 1.08 mmol) followed by sodium iodide (0.16 g, 1.08 mmol). The resultant reaction mixture was stirred at rt for 90 min. The reaction mixture was diluted with H_2O (5 mL), washed with CH_2Cl_2 (2 x 5 mL) and then freeze dried. The residue was purified by HPLC (C_{18} Bondpak reverse phase, 8 x 200 mm, isocratic elution, 100% H_2O, t_R 2.3 min) to give **31** as an off-white solid (27 mg, 50%): $[\alpha]_D^{26}$ -10.7 (*c* 0.2, H_2O); IR (μscope) 3229-2400, 1634, cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 3.41 (dd, 1H, *J* = 16.1, 7.5 Hz C<u>H</u>$_a$H$_b$CHNH), 3.51 (dd, 1H, *J* = 16.1, 5.1 Hz, CH$_a$<u>H</u>$_b$CHNH), 4.12 (dd, 1H, *J* = 7.5, 5.1 Hz, C<u>H</u>NH); 6.51 (s, 1H, C=C<u>H</u>); ^{13}C NMR (D_2O, 75.5 MHz) δ 28.7, 53.8, 104.7, 162.2, 167.1, 169.5, 173.6; HRMS (ES) Calcd for $C_7H_9N_2O_5$ 201.0511, found 201.0516, $[MH]^+$.

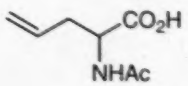
Procedure B: To a solution of **80** (200 mg, 0.584 mmol) in MeCN/H_2O (1:1, 6 mL) was added lithium hydroxide monohydrate (58 mg, 1.29 mmol). The solution was stirred for 17 h at rt and the solvent was removed *in vacuo*. The residue was dissolved in water and extracted with EtOAc (3 x 10 mL). The aqueous layer was acidifed to pH 2 with 6M HCl and extracted with EtOAc (3 x 15 mL). The combined organic extracts were dried over

Na_2SO_4 and concentrated *in vacuo* to give a colourless residue that was dissolved in CH_2Cl_2 (2 mL) and treated with TFA (450 µL, 5.8 mmol). The solution was stirred for 45 min and the solvent removed *in vacuo* to afford a solid which was purified by flash chromatography (30% NH_3 in isopropanol) to give **31** as a white solid (120 mg, 88%). All spectroscopic data obtained for **31** *via* this method are consistent with the previously reported data.



Methyl (2*S*)-2-(*N*-(benzyloxycarbonyl)amino)-4-pentenoate (34).[52] Diazomethane[209] was freshly prepared as follows. To a solution of 2-ethoxyethanol (48 mL) in ether (60 mL) was added KOH (9 g, 161 mmol) in water (15 mL). The mixture was heated to reflux in a smooth jointed distillation apparatus. A solution of Diazald™ (32.1 g, 150 mmol) in ether (120 mL) was added dropwise and the yellow ethereal solution of CH_2N_2 was collected at to 0 °C (**Caution: Diazomethane is potentially explosive !!!! Use only non-ground glass joints for distillation.**) Protected allyl glycine **39** (7.0 g, 28.1 mmol) was dissolved in Et_2O (25 mL) and treated with the freshly prepared diazomethane solution. After 30 min the excess diazomethane was destroyed by the addition of AcOH. The solvent was evaporated under reduced pressure to afford an oil which was purified by flash chromatography (20% EtOAc in hexanes) to give **34** as a colourless oil (6.5 g, 88%): $[\alpha]_D^{26}$ +16.4 (*c* 4.0, $CHCl_3$) (lit[52] $[\alpha]_D^{26}$ +15.3 (*c* 1.6, $CHCl_3$); IR ($CHCl_3$, cast) 3340,

1723, 1525, 739, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.53 (m, 2H, $C\underline{H}_2CHNH$), 3.71 (s, 3H, $OC\underline{H}_3$), 4.45 (dd, 1H, *J* = 7.4, 6.1 Hz, $C\underline{H}NH$), 5.07-5.18 (m, 4H, $C\underline{H}_2$=CH and $C\underline{H}_2Ph$), 5.32 (d, 1H, *J* = 8.0 Hz, $N\underline{H}$), 5.67 (m, 1H, CH_2=$C\underline{H}$), 7.22-7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.6, 52.2, 53.3, 66.9, 119.2, 127.9, 128.1, 128.4, 132.0, 136.2, 155.7, 172.1; HRMS (EI) Calcd for $C_{14}H_{17}NO_4$ 263.1157, found 263.1155, $[M]^+$; Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.98; H, 6.68; N, 5.32.



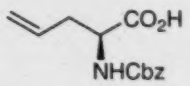
(2*RS*)-2-(*N*-(Acetyl)amino)-4-pentenoic acid (36). The procedure of Black and Wright[97] was adapted for synthesizing this known compound. To a solution of D,L-allylglycine (25 g, 217 mmol) in 2 M NaOH (125 mL) at 0 °C was added acetic anhydride (70 mL, 741 mmol) dropwise over 15 min. The pH was adjusted to 9 by the addition of 2 M NaOH. The reaction mixture was stirred for 1 h at rt and then acidified to pH 2 with conc. HCl. The aqueous solution was then extracted with EtOAc (4 x 300 mL) and the combined organic extracts washed with brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford **36** as a white solid (32.2 g, 94%): IR (EtOAc, cast) 3340, 1723, 1651, 1640 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.04 (s, 3H, $COC\underline{H}_3$), 2.40-2.55 (m, 2H, $C\underline{H}_2CHNH$), 4.80 (dd, 1H, *J* = 8.0, 5.3 Hz, $CH_2C\underline{H}NH$), 5.19 (m, 2H, $C\underline{H}_2$=CH), 5.80 (m, 1H, CH_2=$C\underline{H}$); ^{13}C NMR (D_2O, 75.5 MHz) δ 21.6, 34.9, 52.6, 118.8, 132.7,

174.3, 175.4; HRMS (EI) Calcd for $C_7H_{11}NO_3$ 157.0739, found 157.0741, $[M]^+$; Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.40; H, 7.09; N, 8.82.

37 38

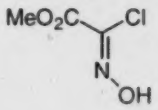
(2*R*)-2-(*N*-(Acetyl)amino)-4-pentenoic acid (37) and (2*S*)-2-Amino-4-pentenoic acid (38).[97] Racemic D,L-allylglycine (61 g, 388 mmol) was dissolved in H_2O (2.7 L) and the pH was adjusted to 7.8 using a saturated solution of LiOH. Porcine kidney acylase I (1.00 g) was added and the solution was stirred at a constant temperature of 37 °C for 16 h. Activated Norit A charcoal (10 g) was added and the mixture was filtered through a bed of Celite™. The yellow solution was then concentrated to a volume of 1 L and applied to a cation exchange resin (Biorad AG 50W-X8, H^+ form, 900 mL bed volume). The column was washed with H_2O until the effluent was neutral and then eluted with 1 M aqueous NH_3. The eluted ammonia solution was evaporated *in vacuo* to afford a white powder which was recrystallized from H_2O/MeOH to give **38** as white crystals (14.8 g, 67%): mp 162-163 °C (lit[97] mp 158-159 °C); $[\alpha]_D^{26}$ -36.4 (*c* 4.0, H_2O) (lit[97] $[\alpha]_D^{26}$ -37.1 (*c* 4.0, H_2O)); IR (Nujol) 3210, 1720, 1642 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.55-2.74 (m, 2H, C$\underline{H}_2$CHNH), 3.80 (dd, 1H, *J* = 7.0, 5.2 Hz, C$\underline{H}$NH)), 5.25 (m, 2H, C$\underline{H}_2$=CH), 5.75 (m, 1H, CH_2=C$\underline{H}$); ^{13}C NMR (D_2O, 75.5 MHz) δ 34.8, 54.0, 120.5, 131.3, 174.1; HRMS (EI) Calcd for $C_5H_9NO_2$ 115.0633, found 115.0633, $[M]^+$; Anal. Calcd for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.17; H, 7.89; N, 12.11.

The eluted aqueous neutral solution was concentrated *in vacuo* to yield a pale yellow solid which was recrystallized from H_2O/MeOH to afford **37** as white crystals (20 g, 65%): mp 104-106 °C (lit[97] mp 104-105 °C); $[\alpha]_D^{26}$ -38.1 (*c* 4.0, EtOH), (lit[97] $[\alpha]_D^{26}$ = -37.8 (*c* 4.0, EtOH)); IR (Nujol) 2904, 1612, 1586 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.04 (s, 3H, $COC\underline{H}_3$), 2.40-2.55 (m, 2H, $C\underline{H}_2CHNH$), 4.80 (dd, 1H, *J* = 8.0, 5.3 Hz, $C\underline{H}NH$), 5.19 (m, 2H, $C\underline{H}_2$=CH), 5.80 (m, 1H, CH_2=$C\underline{H}$); HRMS (EI) Calcd for $C_7H_{11}NO_3$ 157.0739, found 157.0741, $[M]^+$; Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.40; H, 7.09; N, 8.82.

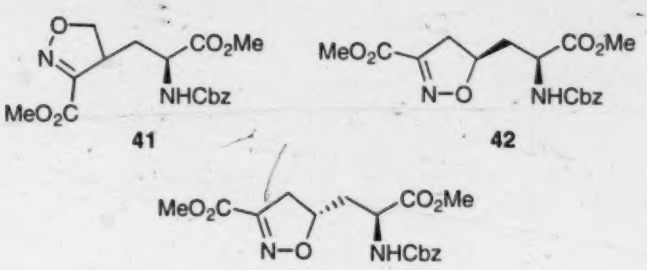


(2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-pentenoic acid (39). The procedure of Cox *et al.*[98] was adapted. To a stirred solution of L-allylglycine **38** (3.28 g, 28.5 mmol) in 0.8 M NaOH (40 mL) at 0 °C was added benzyl chloroformate (7.29 g, 42.7 mmol) dropwise. The pH was adjusted to 9 with the addition of 2 M NaOH. The basic solution was stirred for 2 h at rt, washed with CH_2Cl_2 (2 x 50 mL) and the aqueous layer was acidified to pH 2 with conc. HCl. The acidic solution was then extracted with Et_2O (4 x 50 mL), and the combined ethereal extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to afford **39** as an oil (7.2 g, 90%): $[\alpha]_D^{26}$ +15.1 (*c* 0.88, $CHCl_3$); IR ($CHCl_3$, cast) 3318, 1720, 1521, 738, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.48-2.70 (m, 2H, $C\underline{H}_2NHCH$), 4.47 (dd, 1H, *J* = 13.5, 6.5 Hz, $C\underline{H}NH$), 5.10 (m, 4H, CH=$C\underline{H}_2$, $C\underline{H}_2Ph$), 5.40 (d, 1H, *J* = 7.8 Hz, $N\underline{H}$), 5.61-5.78 (m, 1H, $C\underline{H}$=CH_2), 7.22-7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5

MHz) δ 36.3, 55.1, 67.2, 119.7, 128.1, 128.3, 128.5, 131.8, 136.0, 156.1, 176.2; HRMS (EI) Calcd for $C_{13}H_{15}NO_4$ 249.1001, found 249.0993, $[M]^+$.



Methyl chlorooximidoacetate (40). The title compound was prepared by a modified literature procedure.[99] A solution of methyl glycine hydrochloride (2.0 g, 16 mmol) in water (10 mL) at 0 °C was treated with conc. HCl (5 mL) and then an aqueous solution of $NaNO_2$ (1.10 g, 16 mmol) was added dropwise over 10 min. The resulting solution was stirred at 0 °C for an additional 10 min and then treated with a second equivalent each of HCl and $NaNO_2$. After 45 min, saturated aqueous NaCl (25 mL) was added and the reaction mixture was extracted with Et_2O (3 x 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to afford a light yellow solid that was recrystallized from Et_2O/ hexane to give **40** as colourless prisms (1.20 g, 57%): mp 57-59 °C (lit[99] mp 57-60°C); IR (μscope) 3500-3017, 1720, 1603 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.90 (s, 3H, $OC\underline{H}_3$), 9.29 (br s, 1H, $NO\underline{H}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 54.2, 132.9, 159.2; MS (FAB) *m/z* (relative intensity) M^+ calcd for $C_3H_4NO_3{}^{37}Cl$ 138.9851, found 138.9852 (33%) and calcd for $C_3H_4NO_3{}^{35}Cl$ 136.9880l, found 136.9879 (100); Anal. Calcd for $C_3H_4NO_3{}^{35}Cl$: C, 26.30; H, 2.94; N, 10.22. Found: C, 26.49; H, 2.54; N, 9.91.



41 42

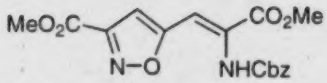
43

Methyl (2*S*,4′*RS*)-2-*N*-(benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-4-yl)-1-propanoate (41), Methyl (2*S*,5′*R*)-2-*N*-(benzyloxycarbonyl)-amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-5-yl)-1-propanoate (42) and Methyl (2*S*,5′*S*)-2-*N*-(benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-5-yl)-1-propanoate (43).[52] To a mixture of methyl *N*-(benzyloxycarbonyl)-L-allylglycinate (**34**) (562 mg, 2.14 mmol) and methyl chlorooximidoacetate (**40**) (876 mg, 6.39 mmol) in Et_2O (7 mL) was added dropwise a solution of Na_2CO_3 (679 mg, 6.40 mmol) in H_2O (5 mL) over 3 h by syringe pump with vigorous stirring. The reaction mixture was then stirred at rt for an additional 4 h and then diluted with Et_2O (15 mL). The phases were separated and the aqueous layer was extracted with Et_2O (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford an oil that was purified by flash chromatography (33% Et_2O in petroleum Et_2O) to afford in order of elution, **41** (5 mg, 0.6%), **42** (264 mg, 34%) and **43** (250 mg, 32%) as colourless oils. For **41**: IR (μscope) 3520-3200, 1722, 1521 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.90-2.03 and 2.11-2.21 (m, 2H, $CHC\underline{H}_2CH$), 3.51-3.59 (m, 1H, $N{=}CC\underline{H}$), 3.73 (s, 3H,

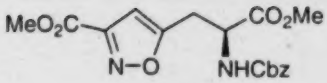
OCH_3), 3.85 (s, 3H, OCH_3), 4.35-4.45 (m, 2H, $CH_2ON{=}C$), 4.53-4.59 (m, 1H, CH_2CHNH), 5.10 (s, 2H, OCH_2Ph), 5.38-5.41 (m, 1H, NH), 7.25-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 34.5, 43.7, 52.2, 52.9, 67.4, 76.8, 128.2, 128.4, 128.6, 136.9, 153.3, 156.0, 171.8; MS (ES) *m/z* (relative intensity) $[M + Na]^+$ 387.1 (100).

For **42**: $[\alpha]_D^{26}$ +73.0 (*c* 1.2, CH_2Cl_2) (lit[52] $[\alpha]_D^{26}$ +95.8 (*c* 1.0, H_2O)); IR (CH_2Cl_2, cast) 3450-3200, 1723, 1525, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.16 (dd, 2H, *J* = 6.5, 6.0 Hz, $CHCH_2CH$), 2.86 (dd, 1H, *J* = 17.6, 7.5 Hz, $CH_aH_b C{=}N$), 3.30 (dd, 1H, *J* = 17.6, 11.2 Hz, $CH_aH_bC{=}N$), 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.43 (dt, 1H, *J* = 6.5, 6.0 Hz, CH_2CHNH), 4.88 (ddt, 1H, *J* = 11.2, 7.5, 7.0 Hz, CH_2CHCH_2), 5.08 (s, 2H, OCH_2Ph), 5.68 (br d, 1H, *J* = 7.0 Hz, NH), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 37.7, 39.1, 51.3, 52.8, 67.2, 80.1, 128.1, 128.3, 128.6, 136.1, 151.2, 155.8, 160.9, 171.7; HRMS (EI) Calcd for $C_{17}H_{21}N_2O$, 365.1349, found 365.1343, $[MH]^+$.

For **43**: $[\alpha]_D^{26}$ -84.6 (*c* 0.6, CH_2Cl_2) (lit[52] $[\alpha]_D^{26}$ -100.9 (*c* 1.0, H_2O)); IR (CH_2Cl_2, cast) 3360-3200, 1724, 1520, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.00 (ddd, 1H, *J* = 14.5, 8.0, 4.5 Hz, $CH_aH_b CHNH$), 2.21 (ddd, 1H, *J* = 14.5, 9.0, 4.5 Hz, CH_aH_bCHNH), 2.84 (dd, 1H, *J* = 17.5, 8.0 Hz, $CH_aH_b C{=}N$), 3.30 (dd, 1H, *J* = 17.5, 11.0 Hz, $CH_aH_bC{=}N$), 3.72 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.53 (ddd, 1H, *J* = 8.5, 8.0, 4.5 Hz, CHNH), 4.88 (dddd, 1H, *J* = 11.0, 9.0, 8.0, 4.5 Hz, CH_2CHCH_2), 5.08 (s, 2H, OCH_2Ph), 5.61 (br d, 1H, *J* = 8.0 Hz, NH), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.7, 39.1, 51.6, 52.7, 52.8, 67.2, 80.6, 128.1, 128.2, 128.5, 136.1, 151.3, 155.9, 160.9, 171.9; HRMS (EI) Calcd for $C_{17}H_{21}N_2O$, 365.1349, found 365.1359, $[MH]^+$.



44

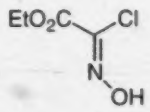


45

Methyl 2-*N*-(benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazole)-2-propenoate (44) and Methyl (2*S*)-*N*-(benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazole)-1-propanoate (45). Active manganese dioxide was prepared according to a known procedure described by Fatiadi *et al.*[106] A solution of a mixture of **42** and **43** (90 mg, 0.248 mmol) in dry benzene (15 mL) and active manganese dioxide (450 mg, five-fold by weight) was heated to reflux for 2 days, with H_2O removal (soxhlet, CaH). The MnO_2 was removed by filtration and washed with benzene (20 mL) and chloroform (50 mL). The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography over silica gel (33% Et_2O in petroleum Et_2O) to give **44** (5 mg, 5.5%) as a solid, **45** (15 mg, 17%) as an oil, and recovered starting material **42** and **43** (45 mg, 50%).

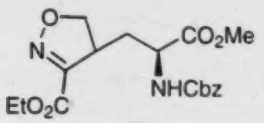
For **44**: IR (CH_2Cl_2, cast) 3319, 1732, 1651, 1575, 1439 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.87 (s, 3H, OC<u>H</u>$_3$), 3.98 (s, 3H, OC<u>H</u>$_3$), 5.20 (s, 2H, OC<u>H</u>$_2$Ph), 6.70 (s, 1H, NHC=C<u>H</u>), 6.90 (s, 1H, C<u>H</u>C=N), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 52.9, 53.3, 68.3, 106.2, 108.9, 128.2, 128.4, 128.5, 128.6, 128.7, 130.1, 135.3, 152.7, 156.6, 159.9, 164.2, 166.9; HRMS (EI) Calcd for $C_{17}H_{16}N_2O_7$ 360.0958, found 360.0954, $[M]^+$; Anal. Calcd for $C_{17}H_{16}N_2O_7$: C, 56.67; H, 4.48; N, 7.77. Found: C, 56.50; H, 4.40; N, 7.46.

For **45**: $[\alpha]_D^{26}$ +31.9 (*c* 0.54, CH_2Cl_2); IR ($CHCl_3$, cast) 3356-3200, 1733, 1652, 1520, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.34 (dd, 1H, *J* = 18.5, 6.0 Hz, C$\underline{H}_a$H$_b$CHNH), 3.45 (dd, 1H, *J* = 18.5, 5.0 Hz, CH$_a$$\underline{H}_b$CHNH), 3.75 (s, 3H, OC$\underline{H}_3$), 3.90 (s, 3H, OC$\underline{H}_3$), 4.70 (m, 1H, C$\underline{H}$NH), 5.20 (s, 2H, OC$\underline{H}_2$Ph), 5.45 (m, 1H, N$\underline{H}$), 6.45 (s, 1H, C$\underline{H}$=C), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 29.7, 52.4, 52.9, 53.1, 67.4, 103.7, 128.1, 128.4, 128.6, 135.9, 155.5, 156.3, 160.3, 170.0, 170.5; HRMS (ES) Calcd for $C_{17}H_{18}N_2O_7Na$ 385.1012, found 385.1012, $[M]^+$.

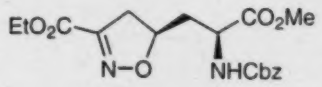


Ethyl chlorooximidoacetate (46).[99] A cooled solution of ethyl glycinate hydrochloride (20 g, 143 mmol) in H_2O (30 mL) was treated with conc. HCl (5 mL) and then an aqueous solution of $NaNO_2$ (9.9 g, 143 mmol) was added dropwise over 10 min. The resulting solution was stirred at 0 °C for 10 min and then treated with a second equivalent each of HCl and $NaNO_2$. After 45 min, saturated aqueous NaCl (25 mL) was added and the mixture was extracted with Et_2O (3 x 75 mL). The combined organic extracts were then dried over Na_2SO_4 and concentrated *in vacuo* to afford a light yellow solid that was recrystallized from Et_2O/ hexane to give **46** as colourless prisms (11.9 g, 55%): mp 75-78 °C (lit[99] mp 76-80 °C); IR (μscope) 3482-3100, 1719, 1618 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.37 (t, 3 H, *J* = 7 Hz, C$\underline{H}_3$), 4.38 (q, 2 H, *J* = 7 Hz, OC$\underline{H}_2$CH$_3$), 8.98 (br s, 1 H,

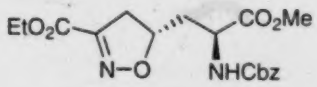
NOH); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.04, 63.8, 133.10, 158.60; MS (FAB) *m/z* (relative intensity) $[M]^+$ 151.8 (85); Anal. Calcd for $C_4H_6NO_3{}^{35}Cl$: C, 31.70; H, 3.99; N, 9.24. Found: C, 31.59; H, 3.76; N, 8.93.



47



48



49

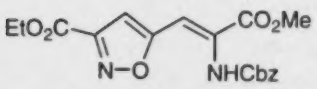
Methyl (2*S*,4′*RS*)-2-*N*-(benzyloxycarbonyl)amino)-3-(3-(ethoxycarbonyl)-2-isoxazolin-4-yl)-1-propanoate (47) and Methyl (2*S*,5′*R*)-2-*N*-(benzyloxycarbonyl)-amino)-3-(3-(ethoxycarbonyl)-2-isoxazolin-5-yl)-1-propanoate (48) and Methyl (2*S*,5′*S*)-2-*N*-(benzyloxycarbonyl)amino)-3-(3-(ethoxycarbonyl)-2-isoxazolin-5-yl)-1-propanoate (49). A mixture of methyl *N*-(benzyloxycarbonyl)-L-allylglycinate (**34**) (588 mg, 2.23 mmol) and ethyl chlorooximidoacetate (**46**) (1.01 g, 6.70 mmol) in Et_2O (5 mL) was treated dropwise with a solution of Na_2CO_3 (710 mg, 6.70 mmol) in H_2O (5 mL) over 3 h by syringe pump with vigorous stirring. The reaction mixture was then stirred at rt for an additional 4 h, then diluted with Et_2O (15 mL), and the phases were separated. The aqueous layer was extracted with Et_2O (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford an oil that was purified by flash chromatography (33% Et_2O in petroleum ether) to afford, in order of elution, **47** (30 mg, 2%), **48** (815 mg, 33%), and **49** (889 mg, 36%) as colourless oils.

For **47**: IR (CH_2Cl_2, cast) 3520-3200, 1722, 1521, 1218 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.35 (t, 3H, *J* = 8.0 Hz, $C\underline{H}_3CH_2O$), 1.90-2.03 and 2.11-2.21 (m, 2H, $CHC\underline{H}_2CH$), 3.51-3.59 (m, 1H, $C\underline{H}C{=}N$), 3.73 (s, 3H, $OC\underline{H}_3$), 3.85 (s, 3H, $OC\underline{H}_3$), 4.28 (q, 2H, *J* = 8.0 Hz, $CH_3C\underline{H}_2O$), 4.35-4.45 (m, 2H, $C\underline{H}_2ON{=}C$), 4.53-4.59 (m, 1H, $CH_2C\underline{H}NH$), 5.10 (s, 2H, $OC\underline{H}_2Ph$), 5.38-5.41 (br s, 1H, N<u>H</u>), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1, 34.4, 43.7, 52.2, 52.8, 62.2, 67.4, 76.6, 128.2, 128.4, 128.6, 135.9, 153.5, 156.3, 160.5, 171.9; MS (ES) *m/z* (relative intensity) $[M + H]^+$ 378.9 (100).

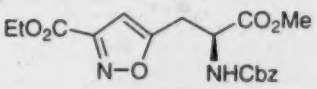
For **48**: $[\alpha]_D^{26}$ +86.9 (*c* 0.4, CH_2Cl_2); IR (CH_2Cl_2, cast) 3360-3200, 1721, 1521, 1437, 1220 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, 3H, *J* = 8.0 Hz, $C\underline{H}_3CH_2O$), 2.13 (t, 2H, *J* = 6.0 Hz, $CHC\underline{H}_2CH$), 2.83 (dd, 1H, *J* = 17.6, 7.6 Hz, $C\underline{H}_aH_bC{=}N$), 3.30 (dd, 1H, *J* = 17.6, 11.0 Hz, $CH_aH_bC{=}N$), 3.72 (s, 3H, $OC\underline{H}_3$), 3.85 (s, 3H, $OC\underline{H}_3$), 4.28 (q, 2H, *J* = 8.0 Hz, $CH_3C\underline{H}_2O$), 4.41 (dt, 1H, *J* = 6.5, 6.0 Hz, $CH_2C\underline{H}NH$), 4.85 (ddt, 1H, *J* = 11.0, 7.6, 6.5 Hz, $CH_2C\underline{H}CH_2$), 5.08 (s, 2H, $OC\underline{H}_2Ph$), 5.75 (d, 1H, *J* = 7.0 Hz, N<u>H</u>), 7.26-7.32 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1, 37.7, 39.1, 51.4, 52.8, 62.1, 67.2, 80.1, 128.1, 128.3, 128.6, 136.0, 151.5, 155.9, 160.5, 171.7; MS (ES) *m/z* (relative intensity) $[M + H]^+$ 378.4 (100); Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 57.14; H, 5.86; N, 7.40. Found: C, 57.46; H, 5.89; N, 7.13.

For **49**: $[\alpha]_D^{26}$ -115.0 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2, cast) 3358-3200, 1721, 1520, 1438, 1220 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, 3H, *J* = 8.0 Hz, $C\underline{H}_3CH_2O$), 2.00 (m, 1H, $C\underline{H}_aH_bCHNH$), 2.21 (ddd, 1H, *J* = 14.4, 9.0, 4.5 Hz, $CH_aC\underline{H}_bCHNH$), 2.84 (dd, 1H, *J* = 17.7, 7.8 Hz, $C\underline{H}_aH_bC{=}N$), 3.30 (dd, 1H, *J* = 17.7, 11.0 Hz, $CH_aC\underline{H}_bC{=}N$), 3.74 (s, 3H, $OC\underline{H}_3$), 4.32 (q, 2H, *J* = 7.1 Hz, $CH_3C\underline{H}_2O$), 4.53 (ddd, 1H, *J* = 8.5, 8.0, 4.5 Hz, $C\underline{H}NH$),

4.88 (dddd, 1H, J = 11.0, 9.0, 7.8, 4.5 Hz, $CH_2C\underline{H}CH_2$), 5.08 (s, 2H, $OC\underline{H}_2Ph$), 5.60 (d, 1H, J = 8.0 Hz, NH), 7.25-7.32 (s, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1, 37.7, 39.2, 51.7, 52.7, 62.2, 67.2, 80.5, 128.1, 128.1, 128.2, 136.1, 151.6, 155.9, 160.5, 171.9; HRMS (ES) Calcd for $C_{18}H_{21}N_2O_7$ 379.1505, found 379.1488, $[MH]^+$.



50



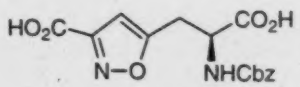
51

Methyl 2-(*N*-(benzyloxycarbonyl)amino)-3-(3-(ethoxycarbonyl)-2-isoxazole)-2-propenoate (50), Methyl (2S)-2-(*N*-(benzyloxycarbonyl)amino)-3-(3-(ethoxy-carbonyl)-2-isoxazole)-1-propanoate (51). Active manganese dioxide was also prepared according to a known procedure described by Fatiadi *et al.*[106] A mixture of isoxazolines **48** and **49** (1.87 g, 4.95 mmol) in dry benzene (95 mL) and active manganese dioxide (9.4 g) was heated to reflux for 3 days with azeotropic removal of H_2O using a Dean-Stark trap. The MnO_2 was removed from the reaction mixture by filtration and washed carefully with benzene and chloroform. The solvent was removed *in vacuo* and the oil was purified by flash chromatography (33% Et_2O in petroleum ether) to give **50** (90 mg, 5%), **51** (210 mg, 11%) and recovered starting materials **48** and **49** (909 mg, 57%) as oils.

For **50**: IR (CH_2Cl_2, cast) 3320, 1732, 1652, 1575, 1436, 1226 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40 (t, 3H, J = 8.0 Hz, $C\underline{H}_3CH_2O$), 3.87 (s, 3H, $OC\underline{H}_3$), 4.45 (q, 2H, J = 8.0 Hz,

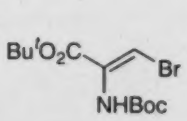
$CH_3C\underline{H}_2O$), 5.20 (s, 2H, $OC\underline{H}_2Ph$), 6.70 (s, 1H, NHC=C$\underline{H}$), 6.90 (s, 1H, C$\underline{H}$C=N), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1, 53.2, 62.1, 68.2, 106.2, 108.9, 128.3, 128.4, 128.5, 128.6, 128.6, 130.1, 135.2, 152.4, 156.6, 159.5, 164.2, 166.8; HRMS (EI) Cacld for $C_{18}H_{19}N_2O_7$ 375.1192, found 375.1181, $[MH]^+$.

For **51**: $[\alpha]_D^{26}$ +35.7 (*c* 1.8, CH_2Cl_2); IR (CH_2Cl_2, cast) 3354-3200, 1732, 1596, 1437, 1223 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (t, 3H, *J* = 8.0 Hz, $C\underline{H}_3CH_2O$), 3.37 (dd, 1H, *J* = 15.0, 5.6 Hz, $C\underline{H}_aH_bCHNH$), 3.45 (dd, 1H, *J* = 15.0, 6.0 Hz, $CH_a\underline{H}_bCHNH$), 3.75 (s, 3H, $OC\underline{H}_3$), 4.40 (q, 2H, *J* = 8.0 Hz, $CH_3C\underline{H}_2O$), 4.68 (m, 1H, C$\underline{H}$NH), 5.20 (s, 2H, $OC\underline{H}_2Ph$), 5.51 (br d, 1H, *J* = 7.1 Hz, N$\underline{H}$), 6.45 (s, 1H, C$\underline{H}$=C), 7.35 (m, 5H, Ph): ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.2, 29.7, 52.4, 53.1, 62.2, 67.4, 103.7, 128.1, 128.4, 128.6, 136.0, 155.6, 156.3, 159.8, 169.9, 170.5; HRMS (ES) Calcd for $C_{17}H_{18}N_2O_7Na$ 377.1349, found 377.1360, $[M]^+$.

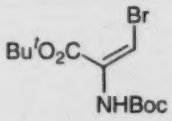


(2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-(carboxy)-2-isoxazole)-1-propanoic acid (52). To a solution of isoxazole **51** (334 mg, 0.888 mmol) in MeCN/H_2O (1:1, 10 mL) was added lithium hydroxide monohydrate (149 mg, 3.55 mmol). The solution was allowed to stir for 18 h at rt, the solvent was evaporated *in vacuo* and the residue taken up in H_2O (5 mL). The solution was washed with EtOAc (2 x 10 mL) and the aqueous layer was acidified to pH 2 using 6 M HCl. The acidic aqueous layer was then extracted with EtOAc (3 x 20 mL) and the combined organic extracts were dried over Na_2SO_4 and

concentrated *in vacuo* to afford a solid which was purified by preparative TLC to afford **52** as white solid (85%, 250 mg): $[\alpha]_D^{26}$ -1.7 (*c* 1.5, acetone); IR (CH_2Cl_2, cast) 3500-2900, 1724, 1596, 1430 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 300 MHz) δ 3.38 (dd, 1H, *J* = 15.6, 8.7 Hz, C$\underline{H}_a$H$_b$CHNH), 3.45 (dd, 1H, *J* = 15.6, 5.0 Hz, CH$_a\underline{H}_b$CHNH), 4.69 (ddd, 1H, *J* = 12.0, 8.7, 5.0 Hz, C$\underline{H}$NH), 5.05 (s, 2H, OC$\underline{H}_2$Ph), 6.65 (s, 1H, C=C$\underline{H}$), 6.82 (m, 1H, N$\underline{H}$), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($(CD_3)_2CO$, 75.5 MHz) δ 29.8, 53.0, 66.9, 104.2, 128.5, 128.6, 129.2, 137.9, 156.9, 157.5, 161.2, 172.0, 172.3; HRMS (ES) Calcd for $C_{15}H_{14}N_2O_7Na$ 357.0699, found 357.0690, $[M]^+$.



64



68

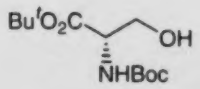
***tert*-Butyl (*Z*)-3-bromo-2-(*N*-(*tert*-butoxycarbonyl)amino)-2-propenoate (64) and *tert*-Butyl (*E*)-3-Bromo-2-(*N*-(*tert*-butoxycarbonyl)amino)-2-propenoate (68).**

The procedure for the preparation of vinyl bromides was used.[124] A solution of **67** (1.14 g, 4.69 mmol) in CH_2Cl_2 (25 mL) was treated dropwise with a solution of bromine until a permanent yellow colour persisted. After stirring at room temperature for 10 min, DBU (0.55 g, 4.93 mmol) was added and the reaction mixture was stirred for an additional 40 min. The white precipitate which had formed was filtered through celite and the solvent removed in vacuo to afford a yellow oil. Purification by flash chromatography (15%

EtOAc in hexanes) afforded isomer **64** as a white solid (700 mg, 46%), and isomer **68** also as a white solid (100 mg, 7%).

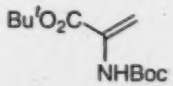
For **64**: IR (CH_2Cl_2, cast) 3320, 1715, 1626, 1254, 1154 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9H, $C(C\underline{H}_3)_3$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 6.08 (br s, 1H, N<u>H</u>), 6.79 (d, 1H, J = 0.8 Hz, C<u>H</u>=C); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.0, 28.2, 81.4, 82.9, 108.5, 133.9, 151.9, 161.2; HRMS (ES) Calcd for $C_{12}H_{20}NO_4NaBr$ 344.0473, found 344.0476, $[M+Na]^+$; Anal. Calcd for $C_{12}H_{20}NO_4Br$: C, 44.73; H, 6.26; N, 4.35. Found: C, 44.64; H, 6.17; N, 4.25.

For **68**: IR (CH_2Cl_2, cast) 3403, 1728, 1700, 1246, 1154 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9H, $C(C\underline{H}_3)_3$), 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 6.93 (s, 1H, N<u>H</u>), 7.48 (s, 1H, C<u>H</u>=C); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.0, 28.1, 81.1, 84.5, 100.7, 129.5, 152.7, 161.9; HRMS (ES) Calcd for $C_{12}H_{20}NO_4NaBr$ 344.0473, found 344.0482, $[M+Na]^+$; Anal. Calcd for $C_{12}H_{20}NO_4Br$: C, 44.73; H, 6.26; N, 4.35. Found: C, 44.89; H, 6.24; N, 4.36.



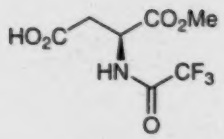
***tert*-Butyl (2*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-3-hydroxypropanoate (66).** To a stirred solution of *N*-((*tert*-butoxycarbonyl)amino)-L-serine (1.4 g, 6.8 mmol) in *N,N'*-dimethylacetamide (85 mL) was added potassium carbonate (25 g, 110 mmol), *tert*-butylbromide (35 mL, 303 mmol), and benzyl triethylammonium chloride (1.7 g, 7.5

mmol). The mixture was then heated at 55 °C for 48 h. The reaction mixture was then poured into H_2O (700 mL) and extracted with EtOAc (300 mL). The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo* to afford a yellow oil which was purified by flash chromatography (20% EtOAc in hexanes) to afford **66** as a white solid (1.42 g, 78%): $[\alpha]_D^{26}$ +5.4 (*c* 0.1, CH_2Cl_2); IR (CH_2Cl_2, cast) 3439, 1719, 1504, 1458, 1250, 1155 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 1.49 (s, 9H, $C(C\underline{H}_3)_3$), 2.40 (s, 1H, O<u>H</u>), 3.86 (d, 2H, *J* = 3.9 Hz, $C\underline{H}_2OH$), 4.22 (m, 1H, C<u>H</u>NHBoc), 5.40 (s, 1H, N<u>H</u>Boc); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.0, 28.3, 56.4, 64.1, 82.7, 156.0, 169.8; MS (ES) *m/z* (relative intensity) $[M + Na]^+$ 284.1 (100); Anal. Calcd for $C_{12}H_{23}NO_5$: C, 55.16; H, 8.87; N, 5.36. Found: C, 54.71; H, 8.87; N, 5.31.



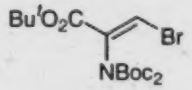
***tert*-Butyl 2-(*N*-(*tert*-butoxycarbonyl)amino)-2-propenoate (67).** To a solution of **66** (0.53 g, 2.00 mmol) in CH_2Cl_2 (7 mL) under argon was added Et_3N (1.13 mL, 8.11 mmol) at 0 °C. The solution was stirred for 5 min and methanesulfonyl chloride (0.17 mL, 2.23 mmol) was added dropwise over 5 min. The solution was allowed to stir at room temperature for 4 h and the solvent was removed *in vacuo* to give a dark orange oil. Flash chromatography (10% EtOAc in hexanes) afforded **67** as a colourless oil (0.35 g, 71%): IR (CH_2Cl_2, cast) 3420, 1733, 1507, 1251, 1155 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H, $C(C\underline{H}_3)_3$), 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 5.59 (d, 1H, *J* = 1.5 Hz, $C\underline{H}_aH_b$=C), 6.03 (m, 1H, $CH_a\underline{H}_b$=C), 7.00 (br s, 1H, N<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.9, 28.3, 80.4,

82.6, 104.0, 132.5, 152.6, 163.1; HRMS (ES) Calcd for $C_{12}H_{21}NO_4Na$ 266.1368, found 266.1366, $[M]^+$.

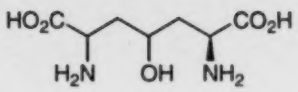


Methyl (2*S*)-2-(*N*-(2,2,2-trifluoroacetyl)amino)-4-oic-1-butanoate (71). The literature procedure for the synthesis of this known compound was adapted.[132] Trifluoroacetic anhydride (22.4 mL, 159 mmol) was added to a stirred suspension of L-aspartic acid (2.5 g, 18.8 mmol) in THF (40 mL) at 0 °C over a 30 min period. The reaction was allowed to warm to rt over 2 h, at which point the solution became homogenous. The solvent was evaporated and the solid was dried *in vacuo* for several hours. MeOH (30 mL) cooled to -15 °C was added and the solution was stirred for 30 min. The methanol was then evaporated *in vacuo* to furnish a mixture of α and β-monoesters as a yellow solid in quantitative yield (4.5 g). This solid (1.0 g, 4.11 mmol) was dissolved in thionyl chloride (10 mL) and heated to reflux for 1.5 h. The solvent was removed *in vacuo* and the traces of thionyl chloride were removed by forming an azeotrope with carbon tetrachloride. The light yellow solid obtained was recrystallized from dry benzene to yield pale yellow crystals of the β-chloro-derivative (0.91 g, 85%). Treatment of this sensitive β-acid chloride (0.50 g, 1.9 mmol) with water resulted in the quantitative formation of **71** (0.46 g, 99%): mp 99-101 °C (lit[132] mp 102-103 °C); $[\alpha]_D^{26}$ +15.8 (*c* 0.6, $CHCl_3$: MeOH (9:1)); IR (μscope) 3288, 1746, 1713, 1187 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.98 (dd, 1H, *J* = 17.9, 4.2 Hz, C<u>H</u>$_a$H$_b$ CH), 3.17 (dd, 1H, *J* = 17.9, 4.0 Hz, CH$_a$<u>H</u>$_b$CH), 3.79 (s, 3H,

OC$\underline{H}_3$), 4.83 (m, 1H, C$\underline{H}$NH), 7.30 (m, 1H, N$\underline{H}$); ^{13}C NMR ((CD$_3$)$_2$CO, 75.5 MHz) δ 35.5, 50.2, 53.3, 116.9, 157.5, 170.5, 171.7; HRMS (ES) Calcd for $C_7H_8NO_5F_3$ 266.0252, found 266.0254, $[M]^+$.



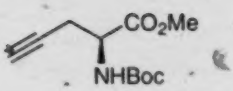
***tert*-Butyl (*Z*)-3-bromo-2-(*N*-(di-*tert*-butoxycarbonyl)amino)-2-propenoate (74).** A solution of vinylbromide **64** (60 mg, 0.19 mmol) in MeCN (5 mL) was treated with di-*tert*-butyl pyrocarbonate (45 mg, 0.21 mmol) and a catalytic amount of DMAP (5 mg, 0.04 mmol). The resultant solution was stirred at room temperature under argon for 2 h, then a second portion of di-*tert*-butyl pyrocarbonate (23 mg, 0.11 mmol) was added and the solution was allowed to stir overnight. The solvent was evaporated to give a pale yellow residue which was purified by flash chromatography (20% EtOAc in hexanes) to give **74** as a colourless oil (66 mg, 85%): IR ($CHCl_3$, cast) 3329, 1765, 1724, 1627, 1254, 1154 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 18H, C(C$\underline{H}_3$)$_3$), 1.48 (s, 9H, C(C$\underline{H}_3$)$_3$), 7.50 (s, 1H, C$\underline{H}$=C); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.9, 81.4, 82.9, 83.1, 121.8, 135.7, 149.2, 161.2; HRMS (ES) Calcd for $C_{17}H_{28}NO_6NaBr$ 444.0998, found 444.0997, $[M+Na]^+$.



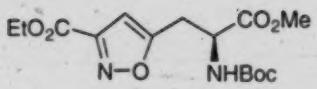
(2*RS*,4*RS*,6*S*)-2,6-diamino-4-hydroxyheptane-1,7-dioic acid (77a-d).

Procedure A: To a solution of **31** (10 mg, 0.043 mmol) in water (4 mL), was added 10% Pd/C (8 mg) or 10% Pt_2O (8 mg). The suspension was stirred under H_2 at atmospheric pressure for 10 h, then filtered through a bed of Celite™ which was subsequently washed with H_2O. The filtrate was evaporated *in vacuo* to give an oily residue which was purified by HPLC (C_{18} Bondpak reverse phase, 8 x 200 mm, 5% MeCN in H_2O, t_R 2.8 min) to give an inseparable mixture of four diastereomers **77a-d** as an oil (7 mg, 79%): IR (μscope) 3500-3060, 1594, 1402 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.82-2.20 (m, 4H, 2 x CH_2), 3.68-3.83 (m, 2H, 2 x $CHNH_2$), 3.86 (s, 1H, OH), 4.06 (m, 1H, CHOH); ^{13}C NMR (D_2O, 125 MHz) δ 37.9, 38.3, 50.7, 53.3, 54.0, 54.2, 66.9, 67.8, 68.9, 174.2, 175.2; HRMS (ES) Calcd for $C_7H_{14}N_2O_5Na$ 229.0800, found 229.0806, $[M+Na]^+$.

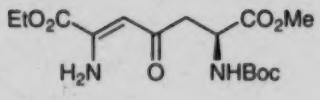
Procedure B: To a solution of **31** (52 mg, 0.22 mmol) in predistilled NH_3(liq) (10 mL), *tert*-butyl alcohol (22 μL, 0.22 mmol) at -78 °C was added sodium (10 mg, 0.47 mmol). The reaction mixture was stirred for 1h and then quenched by the addition of solid NH_4Cl. The ammonia was allowed to evaporate and the solid residue purified by HPLC as described above to afford **77a-d** as an oil (22 mg, 50%). All spectral data obtained *via* this procedure is consistent with previously reported data.



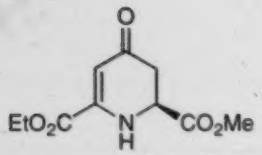
Methyl (2*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-4-pentynoate (79). A modified procedure for the synthesis of this literature compound was used.[140] To MeOH (10 mL) at 0 °C was added thionyl chloride (1.0 mL, 13 mmol) dropwise over 5 min. The solution was stirred for 10 min and L-propargylglycine hydrochloride was added (0.65 g, 4.35 mmol) in one portion. The resulting solution was stirred overnight at room temperature, and the solvent was removed under reduced pressure to give an oily residue. This residue was subsequently dissolved in MeCN (10 mL), and treated with triethylamine (0.73 mL, 5.21 mmol) and di-*tert*-butyl pyrocarbonate (1.14 g, 5.21 mmoL) were added. The reaction mixture was stirred for 2 h at rt, the solvent was evaporated and the resulting residue was suspended in 1 M $NaHSO_4$ (25 mL). The mixture was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic extracts were washed with 1 M $NaHCO_3$ (5 mL), dried over Na_2SO_4, filtered and the solvent removed *in vacuo* to afford an oil which was purified by flash chromatography (10% EtOAc in hexanes) to give **79** as a colourless oil (0.96 g, 95%): $[\alpha]_D^{26}$ -5.0 (*c* 3.0, MeOH) (lit[141] $[\alpha]_D^{26}$ -5.2 (*c* 3.0, MeOH); IR ($CHCl_3$, cast) 3294, 1748, 1715, 1503, 1250, 1163 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 9H, $(C\underline{H}_3)_3$), 2.05 (t, 1H, *J* = 2.6 Hz, $\underline{H}C{\equiv}C$), 2.74 (m, 2H, $C\underline{H}_2CHNH$), 3.79 (s, 3H, $OC\underline{H}_3$), 4.49 (ddd, 1H, *J* = 9.0, 7.9, 4.8 Hz, $C\underline{H}NH$), 5.35 (d, 1H, *J* = 7.9 Hz, $N\underline{H}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.9, 28.3, 51.9, 52.7, 71.6, 77.5, 80.3, 155.1, 171.2; HRMS (ES) Calcd for $C_{11}H_{17}NO_4$ 250.1055, found 250.1057, $[M]^+$.



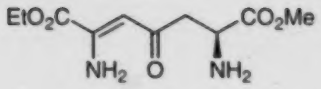
Methyl (2*S*)-2-((*N*-*tert*-butoxycarbonyl)amino)-3-(3-(ethoxycarbonyl)isoxazole)-2-yl-propanoate (80). To a vigorously stirred solution of ethyl chlorooximidoacetate (1.93 g, 12.7 mmol) and methyl *N*-*tert*-butoxycarbonyl-L-propargyl glycinate **79** (0.963 g, 4.24 mmol) in Et_2O (15 mL) was added sodium carbonate (1.35 g, 12.7 mmol) in H_2O (10 mL) via syringe pump over a 5 h period. The reaction mixture was diluted with Et_2O (20 mL), the organic layer was separated, washed with H_2O (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil which was purified by flash chromatography (10-20% EtOAc in hexanes) to give **80** as a white solid (0.94 g, 70%): $[\alpha]_D^{26}$ +43.0 (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$, cast) 3372, 1732, 1715, 1596 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.36 (t, 3H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.40 (s, 9H, $(C\underline{H}_3)_3$), 3.28 (dd, 1H, *J* = 15.5, 5.5 Hz, $C\underline{H}_2CHNH$), 3.41 (dd, 1H, *J* = 15.5, 6.0 Hz, $C\underline{H}_2CHNH$), 3.74 (s, 3H, $OC\underline{H}_3$), 4.39 (q, 2H, *J* = 7.0 Hz, $OC\underline{H}_2CH_3$), 4.60 (m, 1H, $C\underline{H}NH$), 5.20 (m, 1H, $N\underline{H}$), 6.42 (s, 1H, $C\underline{H}$=C(O)CH_2); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.0, 28.1, 29.7, 51.9, 52.8, 62.1, 80.4, 103.4, 154.9, 156.4, 159.8, 170.2, 170.8; HRMS (ES) Calcd for $C_{15}H_{22}N_2O_7Na$ 365.1325, found 365.1327, $[M+Na]^+$; Anal. Calcd for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52.57; H, 6.43; N, 8.09.



1-Ethyl 7-methyl (6*S*)-2-amino-6-(*N*-(*tert*-butoxycarbonyl)amino)-4-oxo-2-heptene-1,7-dioate (81). A modified procedure for reductive ring opening of isoxazoles was used.[141] To a solution of isoxazole **78** (0.45 g, 1.31 mmol), in MeCN (10 mL) under argon was added molybdenum hexacarbonyl (0.22 g, 0.85 mmol) and H_2O (24 μL, 1.31 mmol). The mixture was heated to reflux for 6 h and the solvent was removed *in vacuo* to give a black oil which was purified by flash chromatography (5-25% EtOAc in hexanes) to afford **81** as a light yellow oil (0.285 g, 65%): $[\alpha]_D^{26}$ +77.5 (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, cast) 3431, 1717, 1639, 1593, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.33 (t, 3H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.40 (s, 9H, $(C\underline{H}_3)_3$), 2.93 (dd, 1H, *J* = 17.3, 4.2 Hz, $C\underline{H}_aH_bCHNH$), 3.16 (dd, 1H, *J* = 17.3, 3.9 Hz, $CH_a\underline{H}_bCHNH$), 3.71 (s, 3H, $OC\underline{H}_3$), 4.30 (q, 2H, $OC\underline{H}_2CH_3$), 4.48 (m, 1H, $C\underline{H}NH$), 5.52 (m, 1H, $N\underline{H}$), 5.85 (s, 1H, $C\underline{H}$=C(NH_2)), 8.9 (br s, 2H, $N\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.0, 28.3, 44.2, 50.0, 52.5, 62.7, 79.8, 95.8, 146.3, 155.6, 163.6, 172.5, 198.2; HRMS (ES) Calcd for $C_{15}H_{24}N_2O_7Na$ 367.1481, found 367.1477, $[M+Na]^+$.



82



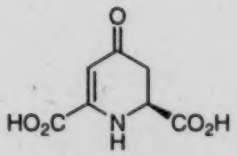
83

2-Ethyl 6-methyl (6*S*)-4-oxo-1,2,3,4-tetrahydropyridine-2,6-dicarboxylate (82) and 1-Ethyl 7-methyl (6*S*)-2,6-diamino-4-oxo-2-heptene-1,7-dicarboxylate (83).

To a solution of vinylogous amide **81** (185 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) was added TFA (413 μL, 5.36 mmol) dropwise over 1 min. The solution was then allowed to stir at rt for 1h. The solvent was evaporated *in vacuo* to give a crude orange oil which was purified by flash chromatography (10% MeOH in EtOAc) to afford **82** (55 mg, 45%) and **83** (60 mg, 46%) as oils. For **82**: $[\alpha]_D^{26}$ +176.6 (*c* 0.9, CH_2Cl_2); IR (CH_2Cl_2, cast) 3362-3200, 1736, 1651, 1438, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.35 (t, 3H, *J* = 7.1 Hz, CH_3CH_2O), 2.69 (dd, 1H, *J* = 15.0, 11.6 Hz, CH_aH_bCHNH), 2.79 (dd, 1H, *J* = 15.0, 6.0 Hz, CH_aH_bCHNH), 3.79 (s, 1H, OCH_3), 4.26-4.40 (m, 3H, CHNH and CH_3CH_2O), 5.76 (s, 1H, C=CH), 6.03 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.0, 38.1, 53.1, 54.6, 63.0, 102.2, 147.6, 162.8, 170.3, 192.3; HRMS (ES) Calcd for $C_{10}H_{15}NO_5Na$ 250.0691, found 250.0693, $[M+Na]^+$.

For **83**: $[\alpha]_D^{26}$ +4.4 (*c* 0.5, MeOH); IR (MeOH, cast) 3500-3298, 1732, 1685, 1444, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.34 (t, 3H, *J* = 7.1 Hz, CH_3CH_2O), 2.90 (dd, 1H, *J* = 17.5, 7.6 Hz, CH_aH_bCHNH), 3.01 (dd, 1H, *J* = 17.5, 3.9 Hz, CH_aH_bCHNH), 3.79 (s, 1H, OCH_3), 3.89 (m, 1H, CHNH), 4.31 (q, 2H, *J* = 7.1 Hz, CH_3CH_2O), 5.87 (s, 1H, C=CH), 6.25 (br s, 2H, NH_2), 9.10 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1,

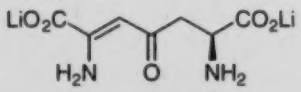
44.7, 51.0, 53.1, 63.3, 95.7, 113.3, 115.6, 117.9, 120.2, 148.2, 161.9, 162.3, 162.6, 162.8, 163.6, 176.1, 199.1; HRMS (ES) Calcd for $C_{10}H_{17}N_2O_5$ 245.1137, found 245.1139, $[MH]^+$. Note: ^{13}C NMR shifts 113.3-120.2 (q, $\underline{C}F_3$) and 161.9-162.8 (q, $CF_3\underline{C}O_2$) are due to trifluoroacetate salt. In addition, compound begins to cyclize in deuterated solvent after several hours.



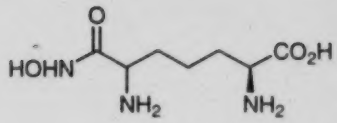
(2*S*)-4-Oxo-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid (84).

To a solution of **82** (100 mg, 0.29 mmol) in MeCN/H_2O (2 mL, 50:50) was added lithium hydroxide monohydrate (40 mg, 0.96 mmol). The solution was stirred at rt overnight and then the solvent was removed *in vacuo*. The resulting residue was dissolved in H_2O (5 mL) and extracted with EtOAc (3 x 5 mL). The aqueous layer was acidified to pH 2 by the dropwise addition of conc. HCl and extracted with EtOAc (3 x 5 mL). Combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow foam which was purified by HPLC (Waters C_{18} resolve 10 µM, gradient elution: 0-12% MeCN in H_2O over 18 min, t_R 4.97 min) to give **84** as a yellow solid (50 mg, 90%): $[\alpha]_D^{26}$ +270.0 (*c* 0.4, H_2O); IR (µscope) 3408, 3093-2200, 1737, 1883, 1605, 1568 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.83 (dd, 1H, *J* = 17.2, 6.5 Hz $CH\underline{H}_aH_bCHNH$), 2.96 (dd, 1H, *J* = 17.2, 7.6 Hz, $CH\underline{H}_aH_bCHNH$), 4.62 (dd, 1H, *J* = 7.6, 6.5 Hz, $CH\underline{H}NH$); ^{13}C NMR (DMSO, 125 MHz) δ

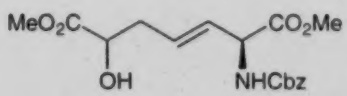
38.8, 54.5, 100.1, 150.6, 165.3, 173.4, 192.3; HRMS (ES) Calcd for $C_7H_8NO_4$ 186.0402, found 186.0404, $[MH]^+$.



(6*S*)-2,6-Diamino-4-oxo-2-heptene-1,7-dioic acid, di-lithium salt (85). To a solution of **83** (233 mg, 0.96 mmol) in H_2O/THF (2 mL, 1:1) was added lithium hydroxide monohydrate (82.4 mg, 2.01 mmol). The solution was allowed to stir at rt for 18 h, the solvent was evaporated and the residue was dissolved in H_2O (8 mL). The solution was extracted with EtOAc (2 x 10 mL) and the aqueous layer was removed *in vacuo* to give a solid residue which was purified using preparative thin layer chromatography (25% NH_4 in isopropanol) to afford **85** as a yellow solid (163 mg, 85%): IR (μscope) 3600-3000, 1601, 1516 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.69 (dd, 1H, J = 17.0, 7.6 Hz C$\underline{H}_a$H$_b$CHNH), 2.74 (dd, 1H, J = 17.0, 9.8 Hz, CH$_2\underline{H}_b$CHNH), 4.28 (dd, 1H, J = 9.8, 7.6 Hz, C$\underline{H}$NH), 5.43 (s, 1H, C=C$\underline{H}$); ^{13}C NMR (D_2O, 125 MHz) δ 37.5, 55.9, 95.0, 159.2, 168.1, 177.3, 196.8; HRMS (ES) Calcd for $C_7H_{11}N_2O_5$ 203.0668, found 203.0662, $[MH]^+$.

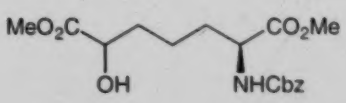


(2*RS*,6*S*)-2,6-Diaminoheptane-1,7-dioic acid, 7-*N*-hydroxyamide (94ab). To a flask flushed with argon was added 10% Pd/C (10 mg) followed by distilled water (5 mL). A solution of **122ab** (77 mg, 0.38 mmol) in H_2O (5 mL) was then added dropwise. The solution was stirred under hydrogen at atmospheric pressure for 2 h. The reaction mixture was filtered through a pad of Celite™ and H_2O was removed *in vacuo*. Purification of the resulting residue by HPLC (C-8 Bondpak reverse phase, 8 x 200 mm, gradient elution: 0-5% MeCN in H_2O (0.1% TFA) over 9 min, t_R 2.7 min) gave a 1:1 mixture of two diastereomers **94a** and **94b** as a solid (27 mg, 60%): IR (μscope) 3500-2900, 1674, 1526, 1437 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.38-2.00 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.80 (m, 1H, $C\underline{H}(NH_2)CONHOH$), 4.00 (m, 1H, $C\underline{H}(NH_2)CO_2H$); ^{13}C NMR (D_2O, 75.5 MHz) δ 18.6, 27.7, 28.6, 49.5, 51.0, 111.5, 113.7, 116.1, 161.2, 164.7, 170.1; HRMS (ES) Calcd for $C_7H_{16}N_3O_4$ 206.1141, found 206.1137, $[MH]^+$.



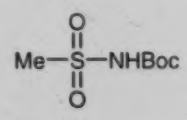
Dimethyl (2*RS*,6*S*)-2-hydroxy-6-(*N*-(benzyloxycarbonyl)amino)-4-heptene-1,7-dioate (97ab). The procedure of Cox *et al.*[99] to synthesize this compound was followed. To a

solution of methyl glyoxylate[159] (4.23 g, 47.9 mmol) in dry CH_2Cl_2 (65 mL) was added $SnCl_4$ (24.9 mL, 212 mmol) at -55 °C over 5 min. The solution was stirred for an additional 10 min and then cooled to -78 °C. A solution of methyl *N*-(Cbz)allylglycinate (**34**) (5.23, 19.9 mmol) in CH_2Cl_2 was added dropwise over 10 min. The white suspension was warmed to -25 °C, stirred for 5 h, poured into 1 M HCl (1 L) and the product was extracted into CH_2Cl_2 (4 x 200 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil which was purified by flash chromatography (40% EtOAc in hexanes) to give a mixture of two diastereomers **97a** and **97b** (5.5 g, 79%) as a colourless oil: IR ($CHCl_3$, cast) 3355, 2953, 1738, 1520 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.34-2.59 (m, 2H, C<u>H</u>$_2$CH=CH), 3.60-3.78 (s, 6H, 2 x OC<u>H</u>$_3$), 4.20-4.26 (m, 1H, C<u>H</u>OH), 4.80-4.89 (m, 1H C<u>H</u>NH), 5.10 (s, 2H, C<u>H</u>$_2$Ph), 5.60-5.65 (m, 2H, C<u>H</u>=CH), 5.72-5.82 (m, 1H, CH=C<u>H</u>), 7.29-7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.9, 52.5, 52.7, 55.5, 67.1, 69.8, 128.0, 128.1, 128.2, 128.5, 136.1, 155.5, 171.0, 174.6; HRMS (EI) Calcd for $C_{17}H_{21}NO_7$ 351.1318, found 351.1319, $[MH]^+$.



Dimethyl (2*RS*,6*S*)-2-hydroxy-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (100ab). The procedure of Cox *et al.*[98] was adapted for the synthesis of this literature compound. A solution of **97ab** (5.50 g, 15.65 mmol) in MeOH (40 mL) was treated with 10% Pd/C (600 mg). The suspension was stirred under H_2 at atmospheric pressure for 20

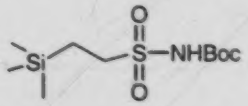
h, then filtered through a bed of Celite™, which was subsequently washed with MeOH. The filtrate was concentrated *in vacuo* to give an oily residue. A solution of this residue (3.40 g, 15.5 mmol) in anhydrous CH_2Cl_2 (40 mL) was treated with pyridine (2.51 mL, 31.01 mmol) and benzyl chloroformate (2.66 mL, 18.6 mmol) at rt. The solution was stirred for 90 min and then added to 1 M HCl (250 mL). The product was extracted into CH_2Cl_2 (4 x 100 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a yellow oil which was purified by flash chromatography (40% EtOAc in hexanes) to give a mixture of two diastereomers **100a** and **100b** as an oil (3.37 g, 61%): IR ($CHCl_3$, cast) 3354, 2953, 1737, 1525, 740, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40-1.95 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.75 (br s, 1H, O<u>H</u>), 3.75 (s, 3H, $OC\underline{H}_3$), 3.76 (s, 3H, $OC\underline{H}_3$), 4.12-4.20 (m, 1H, C<u>H</u>OH), 4.34-4.40 (m, 1H, C<u>H</u>NH), 5.10 (s, 2H, $OC\underline{H}_2Ph$), 5.33-5.39 (br s, 1H, N<u>H</u>), 7.27-7.37 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.6, 32.3, 33.6, 52.4, 67.1, 70.1, 128.1, 128.2, 128.6, 136.3, 155.9, 172.8, 175.4; HRMS (EI) Calcd for $C_{17}H_{23}NO_7$ 353.1474, found 353.1478, $[M]^+$; Anal. Calcd for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.56; N, 3.96. Found: C, 57.78; H, 6.69; N, 3.95.



***tert*-Butyl *N*-(methylsulfonyl)carbamate (102).** The procedure of Campbell and Hart[167] was adapted for the synthesis of this known compound. To a solution of methanesulfonamide (8.0 g, 84 mmol) in anhydrous THF (190 mL) under argon at –55

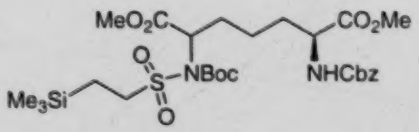
°C was added 2.5 M *n*-BuLi in hexanes (33.6 mL, 84.1 mmol) over a 5-10 min period. The resulting white suspension was cooled to –78 °C with stirring for 50 min, and then potassium hydride (35% in oil) (15.7 g, 132 mmol) was added followed by TMEDA (23 mL, 153 mmol). The mixture was allowed to warm to rt over 90 min. The slurry was then cooled to -78 °C, di-*tert*-butyl pyrocarbonate (23.1 g, 106 mmol) was added over a 2 min period and the reaction mixture was warmed to ambient temperature over 2 h. The slurry was then heated at 55 °C for 16 h. The reaction was quenched by the dropwise addition of *tert*-butyl alcohol (10 mL) at 5 °C. To the resulting yellow suspension was added H_2O (5 mL) over a 10 min period and finally 40 mL of water in one portion. The resultant orange emulsion was further diluted with H_2O (200 mL) and the THF was removed *in vacuo*. The aqueous layer was extracted with EtOAc (3 x 100 mL) and acidified to pH 2-3 using conc. HCl at 0 °C. The product was extracted into CH_2Cl_2 (4 x 250 mL). The combined organic extracts (CH_2Cl_2 and EtOAc) were washed with 3 M HCl (100 mL), brine (75 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a light yellow solid. The solid was dissolved in ether-CH_2Cl_2-MeOH (68:8:1, 85 mL) and hexane (45 mL) was added immediately. The slurry was then refrigerated for 4 h and the white crystals collected by filtration. The mother liquor was recrystallized in a similar fashion to yield a second crop of white crystals. This was combined with the first portion to give **102** (12 g, 75%): mp 110-112 °C (lit[167] mp 107.5-108 °C); IR ($CHCl_3$, cast) 3243, 1741, 1344 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50 (s, 9H, $C(C\underline{H}_3)_3$), 3.25 (s, 3H, $C\underline{H}_3SO_2$), 7.40 (s, 1H, N<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.0, 41.3, 84.5, 149.7;

MS (ES) *m/z* (relative intensity) $[M + Na]^+$ 218.1 (100); Anal. Calcd for $C_6H_{15}NO_4S$: C, 36.91; H, 6.71; N, 7.17. Found: C, 36.85; H, 6.87; N, 7.07.



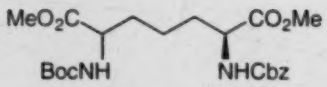
***tert*-Butyl *N*-((2-(trimethylsilyl)ethyl)sulfonyl)carbamate (103).** The procedure of Campbell and Hart[167] was adapted for the synthesis of this known compound. A solution of diisopropylamine (6.40 mL, 45.7 mmol) in THF (35 mL) at –78 °C under argon was treated with 2.5 M *n*-BuLi in hexanes (17.8 mL, 44.5 mmol) over a 5 min period, stirred for 15 min and then treated with a solution of **102** (4.4 g, 22.3 mmol) in THF (15 mL) was added over 10 min. The mixture was stirred for 10 min and then transferred *via* cannula into a solution of (iodomethyl)trimethylsilane in THF (30 mL) at -78 °C over a 15 min period. The solution was stirred for 2 h and then allowed to warm to room temperature over 2 h. The reaction mixture was poured into 0.5 M HCl (210 mL) at 0 °C. The aqueous layer was extracted with CH_2Cl_2 (4 x 100 mL). The combined organic extracts were washed with saturated aqueous sodium bisulfite (50 mL), and brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a yellow oil. The oil was frozen at –78 °C and cold hexane was subsequently added to induce crystallization. The yellow solid that formed was collected by filtration and recrystallized from hexane-ether (9:1) to yield **103** as white crystals (4.0 g, 65%): mp 80-82 °C (lit[167] mp 80-81 °C); IR (μscope) 3263, 1709, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$),

1.02 (m, 2H, C$\underline{H}_2$Si), 1.48 (s, 9H, C(C$\underline{H}_3$)$_3$), 3.30 (m, 2H, C$\underline{H}_2$SO$_2$), 6.82 (s, 1H, N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.1, 10.2, 28.1, 49.3, 84.0, 150.0; MS (ES) *m/z* (relative intensity) $[M + K]^+$ 320.0 (100); Anal. Calcd for $C_{10}H_{23}NO_4SiS$: C, 42.68; H, 8.24; N, 4.98. Found: C, 42.86; H, 8.32; N, 4.91.

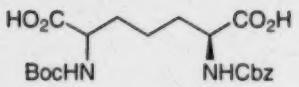


Dimethyl (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl))-(*N*-(2-trimethylsilylethyl-sulfonyl)amino))-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (104ab). The conditions for the Mitsunobu reaction were modified.[162] To a solution of triphenylphosphine (3.78 g, 14.2 mmol), and carbamate **103** (3.78 g, 13.3 mmol) in THF (60 mL) at 0 °C was added hydroxy diester **100ab** (3.10 g, 8.7 mmol) dissolved in THF (20 mL). To the resulting solution DEAD (2.10 mL, 13.3 mmol) was added dropwise over a 5 min period. The solution was stirred for 5 h at rt, concentrated *in vacuo* and purified by flash chromatography (15-40% EtOAc in hexanes) to give a mixture of two diastereomers **104a** and **104b** as a colourless oil (3.90 g, 73%): IR ($CHCl_3$, cast) 3375, 1728, 1519, 1251, 738, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9 H, Si(C$\underline{H}_3$)$_3$), 1.02-2.22 (m, 17H, C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$, C$\underline{H}_2$Si, C(C$\underline{H}_3$)$_3$), 3.40-3.60 (m, 2H, C$\underline{H}_2$SO$_2$), 3.71 (s, 6H, 2 x OC$\underline{H}_3$), 4.28-4.42 (m, 1H, C$\underline{H}$NH), 4.71 (dd, 1H, *J* = 9.0, 5.3 Hz, C$\underline{H}$N), 5.08 (s, 2H, OC$\underline{H}_2$Ph), 5.28-5.36 (m, 1H, N$\underline{H}$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.7, 21.9, 27.9, 31.8, 32.2, 50.8, 52.5, 53.7, 58.7, 58.9, 66.9, 85.1, 128.0,

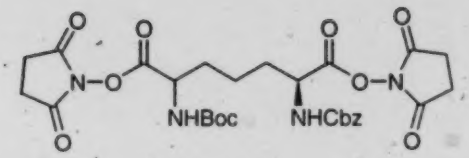
128.5, 136.4, 150.9, 155.9, 170.4, 172.9; MS (FAB) *m/z* (relative intensity) $[M + H]^+$ 617.2 (100); Anal. Calcd for $C_{27}H_{44}N_2O_{10}SiS$: C, 52.58; H, 7.19; N, 4.54. Found: C, 52.33; H, 7.29; N, 4.52.



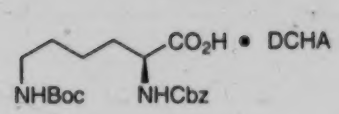
Dimethyl (2*RS*,6*S*)-2-(*N*-(*tert*-butoxycarbonyl)-amino)-6-(*N*-(benzyloxycarbonyl)-amino)heptane-1,7-dioate (105ab). To a solution of **104ab** (635 mg, 1.03 mmol) in THF (40 mL) was added a 1 M solution of TBAF in THF (3.1 mL, 3.10 mmol). The resulting orange solution was stirred for 40 min and diluted with 130 mL of Et_2O. The organic layer was separated, washed with H_2O (4 x 150 mL) followed by saturated aqueous $NaHCO_3$ (50 mL) and was then dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the residue by flash chromatography (25% EtOAc in hexanes) afforded a mixture of two diastereomers **105a** and **105b** as a colourless oil (795 mg, 99%): IR ($CHCl_3$, cast) 3346, 2953, 1741, 1520, 1249, 739, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H, $C(CH_3)_3$), 1.58-1.90 (m, 6H, $CH_2CH_2CH_2$), 3.71 (m, 6H, 2 x OCH_3), 4.24 (m, 1H, CHNHBoc), 4.33 (m, 1H, CHNHCbz), 5.02 (m, 1H, NHBoc), 5.09 (s, 2H, OCH_2Ph), 5.36 (m, 1H, NHCbz), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.2, 28.3, 31.9, 32.3, 52.4, 53.6, 57.1, 58.0, 67.1, 80.0, 128.1, 128.2, 128.5, 135.2, 155.7, 156.1, 172.7, 173.0; MS (ES) *m/z* (relative intensity) $[M + Na]^+$ 475.2 (100); Anal. Calcd for $C_{22}H_{32}N_2O_8$: C, 58.40; H, 7.13; N, 6.19. Found: C, 58.76; H, 7.36; N, 5.93.



(2*RS*,6*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-6-(*N*-(benzyloxycarbonyl)amino)-heptane-1,7-dioic acid (106ab). The procedure of Cox *et al.* was adapted.[98] To a suspension of **105ab** (708 mg, 1.57 mmol) in MeCN/H_2O (50:50, 20 mL) was added lithium hydroxide monohydrate (131 mg, 3.13 mmol). The mixture was stirred for 2.5 h at rt, diluted with 30 mL of H_2O, and washed with EtOAc (2 x 25 mL). The aqueous layer was acidified to pH 2 with 3 M HCl and the solution was extracted with Et_2O (3 x 35 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated *in vacuo* to afford a mixture of two diastereomers **106a** and **106b** as a colourless oil (631 mg, 95%): IR ($CDCl_3$, cast) 3320, 1721, 1530, 739, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H, $C(CH_3)_3$), 1.65-1.90 (m, 6H, $CH_2CH_2CH_2$), 4.29 (m, 1H, CHNHBoc), 4.38 (m, 1H, CHNHCbz), 5.02-5.20 (m, 3H, NHBoc, OCH_2Ph), 5.45 (m, 1H, NHCbz), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.1, 28.3, 31.9, 32.3, 52.4, 53.6, 67.1, 80.0, 128.1, 128.2, 128.5, 135.2, 155.7, 156.1, 172.7, 173.0; MS (FAB) *m/z* (relative intensity) $[M + H]^+$ 425.2 (100).

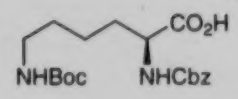


Di-(2,5-dioxopyrrolidin-1-oxy) (2*RS*,6*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (107ab). To a solution of **106ab** (100 mg, 0.24 mmol) and *N*-hydroxysuccinimide (57 mg, 0.49 mmol) in dry 1,2-dimethoxyethane (10 mL) cooled to 0 °C was added dicyclohexylcarbodiimide (105 mg, 0.51 mmol) with stirring. The reaction mixture was stirred at 0-5 °C for 2 h and then allowed to stand in the refrigerator overnight. The *N,N'*-dicyclohexylurea was filtered off and the crystals were washed with EtOAc (15 mL). The combined organic extracts were concentrated *in vacuo* to afford a crude oil which was dissolved in EtOAc (10 mL) and triturated with petroleum ether to give a second crop of the dicyclohexylurea which was filtered. Evaporation of the solvent followed by purification of the product using flash chromatography (30% petroleum ether in EtOAc) afforded a mixture of two diastereomers **107a** and **107b** as a white solid (27 mg, 50%): IR ($CDCl_3$, cast) 3347, 1817, 1785, 1740, 1515, 1455, 1251, 910, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 1.65-1.90 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.60-2.90 (m, 8H, 2 x $COC\underline{H}_2C\underline{H}_2CO$), 4.60-4.80 (m, 2H, C<u>H</u>NHBoc, C<u>H</u>NHCbz), 5.02-5.20 (m, 3H, N<u>H</u>Boc, $OC\underline{H}_2Ph$), 5.60 (m, 1H, N<u>H</u>Cbz), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.2, 25.0, 25.6, 28.3, 51.9, 67.4, 128.2, 128.3, 128.6, 136.1, 154.9, 155.8, 168.1, 168.3, 168.6; HRMS (ES) Calcd for $C_{28}H_{34}N_4O_{12}Na$ 641.2071, found 641.2071, $[M+Na]^+$.

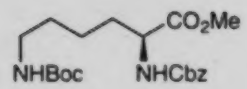


(2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-(*N*-(*tert*-butoxycarbonyl)amino) hexanoic acid dicyclohexylamine salt (112). The procedure of Pope and coworkers[168] was adapted to synthesize this known compound. To a solution of *N*-α-Cbz-L-lysine (2.03 g, 7.24 mmol) in a mixture of dioxane (20 mL), H_2O (10 mL) and 1 M NaOH (10 mL) at 0 °C was added di-*tert*-butyl pyrocarbonate (1.74 g, 7.96 mmol). Stirring was continued at rt for 5 h. The reaction mixture was then concentrated *in vacuo* to 15 mL and acidified to pH 2 with 1 M $KHSO_4$. The aqueous phase was extracted with EtOAc (3 x 25 mL). The combined organic extracts were washed with water (2 x 30 mL), dried over Na_2SO_4 and the solvent evaporated under reduced pressure to afford a colourless oil. The oil was dissolved in EtOAc (30 mL) and DCHA (1.45 mL, 7.24 mmol) was added. The solution was cooled to -20 °C, and Et_2O (30 mL) was added. The white crystals which formed were filtered and dried *in vacuo* to afford **112** (3.67 g, 90%): $[\alpha]_D^{26}$ +9.5 (*c* 1.0, $CHCl_3$): IR ($CDCl_3$, cast) 3347, 2934, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H, $C(CH_3)_3$), 1.10-2.05 (m, 26H, $CH_2CH_2CH_2$ of lysine, and 10 x CH_2's of C_6H_{11} ring), 2.88-3.00 (m, 2H, 2 x HNCH's), 3.01-3.12 (m, 2H, CH_2NHBoc), 4.08 (m, 1H, CHNHCbz), 4.60 (m, 1H, NHBoc), 5.02 (s, 2H, OCH_2Ph), 5.65 (m, 2H, $NH(C_6H_{11})_2$ and NHCbz), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.6, 24.7, 25.2, 28.4, 29.1, 29.7, 32.4, 40.6, 52.5, 55.7, 66.3, 78.9, 127.9, 128.0, 128.4, 136.9, 155.9, 176.1; MS (ES) *m/z*

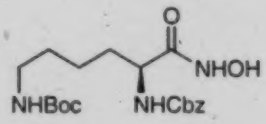
(relative intensity) $[M + H]^+$ 562.4 (100); Anal. Calcd for $C_{31}H_{43}N_3O_8$: C, 66.28; H, 9.15; N, 7.48. Found: C, 66.16; H, 9.44; N, 7.44.



(2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-(*N*-(*tert*-butoxycarbonyl)amino) hexanoic acid (113).[169] To a stirred suspension of **112** (413 mg, 0.735 mmol) in EtOAc (5 mL) was added 0.5 M $KHSO_4$ (2 mL) and stirring was continued at room temperature until all solids had dissolved. The resultant two layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with H_2O (2 x 10 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **113** as a colourless oil (275 mg, 99%); $[\alpha]_D^{26}$ +15.2 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$, cast) 3300, 2934, 1709, 755, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 1.10-2.05 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.01-3.12 (m, 2H, $C\underline{H}_2NHBoc$), 4.08 (m, 1H, $C\underline{H}NHCbz$), 4.60 (m, 1H, $N\underline{H}Boc$), 5.02 (s, 2H, $OC\underline{H}_2Ph$), 5.65 (m, 1H, $N\underline{H}Cbz$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.2, 28.4, 29.7, 32.4, 40.6, 55.7, 66.3, 78.9, 127.9, 128.0, 128.4, 136.9, 155.9, 176.1; HRMS (ES) Calcd for $C_{19}H_{28}N_2O_6Na$ 403.1845, found 403.1843, $[M+Na]^+$.

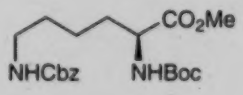


Methyl (2*S*)-2-(*N*-(benzyloxycarbonyl)amino)-6-(*N*-(*tert*-butoxycarbonyl)amino) hexanoate (114). The procedure for the preparation of this known compound was modified.[171] To a solution of **113** (357 mg, 0.938 mmol) in Et_2O (20 mL) was added freshly distilled ethereal diazomethane[209] dropwise until a yellow colour persisted. After 30 min, the excess diazomethane was destroyed by the dropwise addition of AcOH. The solvent was evaporated *in vacuo* to afford an oil which was purified by flash chromatography (20 % EtOAc in hexanes) to give **114** as a colourless oil (355 mg, 96%): $[\alpha]_D^{26}$ +4.8 (*c* 1.0, $CHCl_3$); IR ($CDCl_3$ cast) 3347, 2932, 1700, 1525, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.19-1.84 (m, 15H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 3.15 (m, 1H, $C\underline{H}_2NHBoc$), 3.70 (s, 3H, $CO_2C\underline{H}_3$), 4.38 (m, 1H, $C\underline{H}NH$), 4.55 (s, 1H, $N\underline{H}Boc$), 5.06 (m, 2H, $OC\underline{H}_2Ph$), 5.35 (m, 1H, $N\underline{H}Cbz$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.4, 28.4, 29.6, 32.2, 40.1, 52.4, 53.8, 67.1, 79.2, 128.2, 128.2, 128.6, 136.3, 156.0, 156.1, 172.9; HRMS (ES) Calcd for $C_{20}H_{30}N_2O_6Na$ 417.2002, found 417.2008, $[M+Na]^+$.



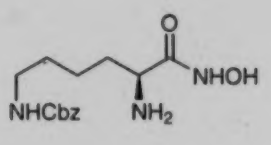
(2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-(*N*-(*tert*-butoxycarbonyl)amino)hexanoic acid, *N*-hydroxyamide (115). To a suspension of **114** (104 mg, 0.26 mmol) in MeOH (1

mL) was added 1 M hydroxylamine in methanol (400 µL, 0.40 mmol). The solution was stirred for 3 days at rt. The solvent was evaporated *in vacuo* to give a residue which was purified by flash chromatography (10% MeOH in EtOAc) to give **115** as a solid (5 mg, 5%): $[\alpha]_D^{26}$ -8.7 (*c* 1.0, MeOH); IR (µscope) 3312, 3037, 2451, 1690, 1675, 1633, 1530, 1433, 1281 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.25-1.80 (m, 15H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 3.00 (m, 2H, $C\underline{H}_2CHNHCbz$), 3.98 (m, 1H, $C\underline{H}NHCbz$), 5.06 (s, 2H, $OC\underline{H}_2Ph$), 7.27-7.38 (m, 5H, Ph); ^{13}C NMR (CD_3OD, 125 MHz) δ 24.0, 28.8, 30.5, 33.0, 41.0, 48.5, 54.2, 67.7, 79.9, 128.8, 129.0, 129.4, 138.1, 158.3, 158.5, 171.6; HRMS (ES) Calcd for $C_{19}H_{29}N_3O_6Na$ 418.1954, found 418.1964, $[M+Na]^+$.

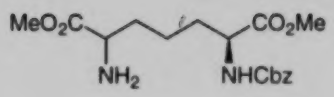


Methyl (2*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-6-(*N*-(benzyloxycarbonyl)amino) hexanoate (116). The procedure was modified[172] for the preparation of this known compound. To a solution of *N*-α-Boc-*N*-ε-Cbz-L-lysine (0.20 g, 0.53 mmol) in Et_2O (10 mL) was added dropwise freshly distilled ethereal diazomethane[209] until a yellow colour persisted. After 30 min, the excess diazomethane was destroyed by the dropwise addition of AcOH. The solvent was evaporated *in vacuo* to afford an oil which was purified by flash chromatography (20 % EtOAc in hexanes) to give **116** as a colourless oil (190 mg, 93%): IR ($CDCl_3$ cast) 3342, 2951, 1710, 1526, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.19-1.84 (m, 15H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 3.15 (m, 1H, $C\underline{H}_2NHBoc$), 3.70 (s, 3H,

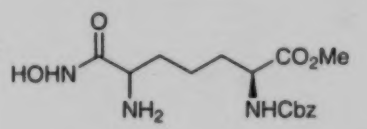
CO_2CH_3), 4.26 (m, 1H, CHNH), 4.82 (s, 1H, NHBoc), 5.06 (m, 2H, OCH_2Ph), 7.26-7.38 (m, 5H, Ph); HRMS (ES) Calcd for $C_{20}H_{30}N_2O_6Na$ 417.2002, found 417.2010, $[M+Na]^+$.



(2*S*)-2-Amino-6-(*N*-(benzyloxycarbonyl)amino)hexanoic acid, *N*-hydroxyamide (119). A 1 M solution of hydroxylamine in MeOH was prepared by the addition of KOH (1.12 g, 20 mmol) to hydroxylamine hydrochloride (1.39, 20 mmol) in MeOH (20 mL) and subsequent removal of the potassium chloride by filtration. To a solution of methyl *N*-ε-(benzyloxycarbonyl)amino)-L-lysinate (574 mg, 1.94 mmol) in MeOH (10 mL) was added 1 M hydroxylamine (6 mL, 6 mmol). The resulting solution was allowed to stir for 24 h at rt and the MeOH was evaporated *in vacuo* to give a white residue which was recrystallized from MeOH to afford white crystals of **119** (210 mg, 36%): mp (dec. > 180 °C); $[\alpha]_D^{26}$ +8.3 (*c* 1.0, DMSO); IR (μscope) 3339, 3034, 1691, 1611, 1535, 1462, 1256 cm^{-1}; ^1H NMR ($(CD_3)_2NCOD$, 300 MHz) δ 1.25-1.65 (m, 6H, $CH_2CH_2CH_2$), 3.10 (m, 3H, CH_2NHCbz, $CHNH_2$), 5.06 (s, 2H, OCH_2Ph), 7.10 (m, 1H, NHCbz), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($(CD_3)_2SO$, 75.5 MHz) δ 22.7, 29.6, 35.1, 40.1, 52.6, 65.1, 127.7, 128.3, 137.3, 156.0, 171.9; HRMS (EI) Calcd for $C_{14}H_{21}N_3O_4$ 295.1532, found 295.1521, $[M]^+$.

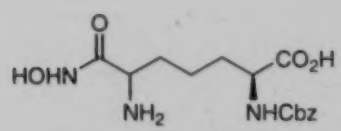


Dimethyl (2*RS*,6*S*)-2-amino-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (120ab). To a solution of dimethyl ester **105ab** (1.42 g, 3.15 mmol) in CH_2Cl_2 (10 mL) was added dropwise TFA (12 mL, 150 mmol). The solution was stirred for 1 h and the solvent was evaporated *in vacuo*. The resultant oil was purified by HPLC (C_8 Bondpak reverse phase, 8 x 200 mm, gradient elution: 0-30% MeCN in H_2O containing 0.1% TFA over 15 min) to give a mixture of two diastereomers **120a** and **120b** as a colourless foam (t_R 12.8 min, 1.05 g, 98%): IR (CH_2Cl_2 cast) 3500-2900, 1747, 1695, 1531, 1439 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.58-1.98 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 3.81 (s, 3H, $OC\underline{H}_3$), 4.01 (m, 1H, $C\underline{H}NH_2$), 4.19 (m, 1H, $C\underline{H}NHCbz$), 5.09 (s, 2H, $OC\underline{H}_2Ph$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 22.4, 30.9, 32.1, 52.8, 53.6, 53.7, 55.0, 67.7, 128.8, 129.0, 129.5, 138.1, 158.7, 170.8, 174.2; HRMS (ES) Calcd for $C_{17}H_{24}N_2O_6$ 353.1713, found 353.1716, $[MH]^+$.



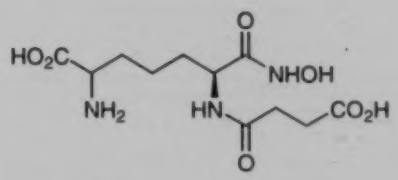
Methyl (2*RS*,6*S*)-2-amino-6-(*N*-(benzyloxycarbonyl)amino)heptane-7-oate, 1-*N*-hydroxyamide (121ab). A procedure similar to that for the preparation of **115** was

utilized. To a solution of **120ab** (1.20 g, 3.41 mmol) in MeOH (8 mL) was added a 1 M solution of hydroxylamine (9.4 mL, 9.4 mmol) in MeOH. The solution was allowed to stir for 1 day at rt, and the solvent was removed *in vacuo* to give a residue. This residue was purified by HPLC (C_8 Bondpak reverse phase, 8 x 200 mm, gradient elution: 0-30% MeCN in H_2O containing 0.1% TFA over 15 min) to afford a mixture of two diastereomers **121a** and **121b** as a foam (t_R 9.75 min, 0.72 g, 65%) and recovered starting material **120ab** (t_R 12.9 min, 0.30 g, 25%): IR (μscope) 3500-2900, 1679, 1529, 1438, 1203 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.40-1.98 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.65 (s, 1H, $C\underline{H}NH_2$), 3.70 (s, 3H, $OC\underline{H}_3$), 4.18 (m, 1H, $C\underline{H}NHCbz$), 5.09 (m, 2H, $OC\underline{H}_2Ph$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 23.4, 33.7, 36.0, 54.2, 54.2, 57.7, 67.4, 116.3, 120.1, 128.8, 128.9, 129.4, 138.3, 158.3, 162.9, 163.4, 170.1, 180.0; HRMS (ES) Calcd for $C_{16}H_{24}N_3O_6$ 354.1665, found 354.1662, $[MH]^+$.

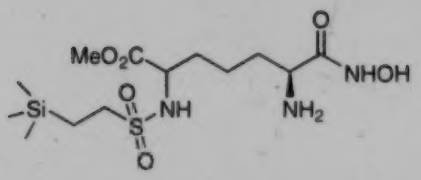


(2*RS*,6*S*)-2-Amino-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioic acid, 1-*N*-hydroxyamide (122ab). To a solution of **121ab** (0.494 g, 1.40 mmol) in MeCN/H_2O (1:1, 15 mL) was added lithium hydroxide monohydrate (0.117 g, 41.96 mmol). The solution was allowed to stir for 16 h at rt. The reaction mixture was then concentrated and the residue was dissolved in water. The aqueous solution was washed with EtOAc (2

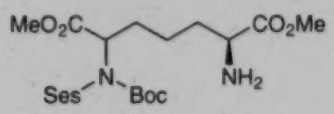
x 20 mL). The aqueous layer was then acidified to pH 2 with conc. HCl and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a solid, which was purified by HPLC (C_8 Bondpak reverse phase, 8 x 200 mm, gradient elution: 0-25% MeCN in H_2O containing 0.1% TFA over 18 min) to give a mixture of two diastereomers **122a** and **122b** as a foam (t_R 7.3 min, 0.81 g, 85%): IR (μscope) 3500-2900, 1679, 1529, 1438 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 1.40-1.99 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.65 (m, 1H, $C\underline{H}NH_2$), 4.18 (m, 1H, $C\underline{H}NHCbz$), 5.09 (s, 2H, $OC\underline{H}_2Ph$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 22.5, 32.1, 32.3, 52.4, 54.9, 67.8, 128.8, 129.1, 129.5, 138.1, 158.8, 167.0, 175.5; HRMS (ES) Calcd for $C_{15}H_{22}N_3O_6$ 340.1509, found 340.1506, $[MH]^+$.



(2*RS*,6*S*)-2-Amino-6-(*N*-(3-carboxyproponyl)amino)heptane-1,7-dioic acid, 7-*N*-hydroxyamide (130ab). Reaction of **169ab** (2.5 mg, 63 μmol) with TFA (5 μL, 0.062 mmol) in CH_2Cl_2(500 μL) gave a mixture of diastereomers **130a** and **130b** (2.5 mg, 99%) as a solid: IR (μscope) 3310, 2931, 1667, 1536 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.40-2.00 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.50-2.70 (m, 4H, $C\underline{H}_2C\underline{H}_2CO_2H$), 3.75 (m, 1H, $C\underline{H}NH_2$), 4.28 (m, 1H, $C\underline{H}NH$); ^{13}C NMR (D_2O, 75.5 MHz) δ 20.8, 29.1, 29.6, 30.0, 30.4, 51.4, 53.7, 170.5, 173.4, 175.1, 177.1; HRMS (ES) Calcd for $C_{11}H_{19}N_3O_7Na$ 328.1121, found 328.1119, $[M+Na]^+$.

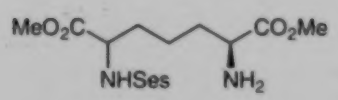


1-Methyl (2*RS*,6*S*)-6-amino-2-(*N*-(2-trimethylsilylethylsulfonyl)amino)heptane-1,7 dioate, 7-*N*-hydroxyamide (131ab). To a solution of **134ab** (0.24 g, 0.63 mmol) in MeOH (5 mL) was added 1 M hydroxylamine in MeOH (1.8 mL, 1.90 mmol). The solution was stirred for 2 days at rt, and the solvent evaporated *in vacuo* to give a residue. This residue was purified by reverse phase HPLC (C_{18} Bondpak, 8 x 200 mm, gradient elution, 0-35% MeCN in H_2O containing 0.1% TFA over 20 min) to give a mixture of two diastereomers **131a** and **131b** (t_R 16.1 min, 0.12 g, 50%) as a solid: IR (μscope) 3400, 3181, 1743, 1673, 1518, 1435, 1252 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 1.01 (m, 2H, $C\underline{H}_2Si$), 1.48-2.10 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.95 (m, 2H, $C\underline{H}_2SO_2$), 3.65 (m, 1H, $C\underline{H}NH_2$), 3.73 (s, 3H, $OC\underline{H}_3$), 4.01 (m, 1H, $C\underline{H}NHSO_2$); ^{13}C NMR (CD_3OD, 125 MHz) δ -2.0, 11.3, 22.2, 31.8, 33.4, 50.5, 52.4, 52.9, 56.8, 166.9, 174.0; HRMS (ES) Calcd for $C_{13}H_{29}N_3O_6SiSNa$ 406.1444, found 406.1442, $[M+Na]^+$.



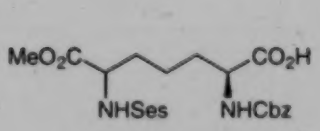
Dimethyl (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl))-*N*-(2-trimethylsilylethylsulfonyl-amino))-6-aminoheptane-1,7-dioate (133ab). To a solution of **104ab** (1.01 g, 1.63

mmol) in MeOH (25 mL) was added 10% Pd/C (100 mg), and the reaction mixture was stirred under H_2 at atmospheric pressure for 8 h. The solution was then filtered through a bed of Celite™, and concentrated *in vacuo* to give an oil which was purified by flash chromatography (50% EtOAc/ 2% aq. NH_4 in hexanes) to afford a mixture of two diastereomers **133a** and **133b** (0.64 g, 81%) as a colourless oil: IR ($CHCl_3$, cast) 3350, 3300, 1733, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 0.90-2.20 (m, 17H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2Si$, $C(C\underline{H}_3)_3$), 3.50 (m, 3H, $C\underline{H}NH_2$, $C\underline{H}_2SO_2$), 3.63 (s, 3H, $OC\underline{H}_3$), 3.70 (s, 3H, $OC\underline{H}_3$), 4.71 (m, 1H, $C\underline{H}N$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.6, 22.5, 27.9, 30.1, 34.4, 50.7, 51.9, 52.4, 54.1, 58.9, 60.0, 84.9, 150.9, 170.4, 176.3; HRMS (EI) Calcd for $C_{19}H_{38}N_2O_6SiS$ 482.2118, found 482.2113, $[M]^+$.

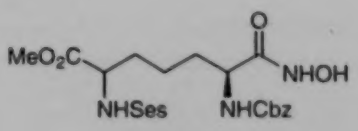


Dimethyl (2*RS*,6*S*)-2-(*N*-(2-trimethylsilylethylsulfonyl)amino)-6-aminoheptane-1,7-dioate (134ab). To a solution of **133ab** (0.38 g, 0.80 mmol) in CH_2Cl_2 (10 mL) was added TFA (0.62 mL, 8.00 mmol), and the solution was stirred for 2 h. The solvent was removed *in vacuo* to give an oil, which was purified by reverse phase HPLC (C_8 Bondpak, 8 x 200 mm, gradient elution: 0-35% MeCN in H_2O containing 0.1% TFA over 20 min) to give a mixture of two diastereomers **134a** and **134b** (t_R 10.7 min, 0.28 g, 90%) as a colourless oil: IR ($CHCl_3$ cast) 3350, 3300, 1732, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9 H, $Si(C\underline{H}_3)_3$), 0.90-2.20 (m, 8H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2Si$), 2.90 (m, 3H,

C<u>H</u>NH$_2$, C<u>H</u>$_2$SO$_2$), 3.63 (s, 3H, OC<u>H</u>$_3$), 3.70 (s, 3H, OC<u>H</u>$_3$), 4.01 (m, 1H, C<u>H</u>NHSO$_2$), 5.80 (br m, 1H, NHSO$_2$); ^{13}C NMR (CDCl$_3$, 75.5 MHz) δ -2.1, 10.1, 30.0, 34.4, 35.0, 49.8, 52.7, 53.5, 55.6, 169.9, 172.7; HRMS (EI) Calcd for $C_{13}H_{31}N_2O_6SiS$ 383.1672, found 383.1616, $[MH]^+$.



136ab



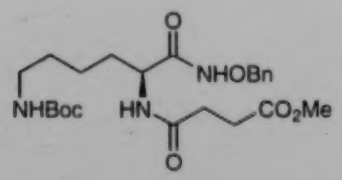
138ab

1-Methyl (2*RS*,6*S*)-2-(*N*-(2-trimethylsilylethylsulfonyl)amino)-6-(*N*-benzyloxycarbonyl)amino)heptane-1,7-dioic acid (136ab) and 1-Methyl (2*RS*,6*S*)-2-(*N*-(2-trimethylsilylethylsulfonyl)amino)-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioic acid, 7-*N*-hydroxyamide (138ab).

To a solution of **131ab** (21 mg, 0.055 mmol) in CH_2Cl_2 (5mL) was added pyridine (8.9 μL, 0.11 mmol) and benzyl chloroformate (9.5 μL, 0.066 mmol). The solution was stirred at 0 °C for 2.5 h, then transferred *via* cannula to a saturated aqueous NH_2OH solution (30 mL). The suspension was stirred for 20 min and acidified to pH 2 using 6 M HCl. The mixture was extracted with EtOAc (3 x 15 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated *in vacuo* to give a residue. This was purified by flash chromatography (30-70% EtOAc in hexanes, 0.1% AcOH) to give a mixture of two diastereomers **136a** and **136b** (6 mg, 21%) as an oil and a mixture of two diastereomers **138a** and **138b** (6 mg, 21%) as a colourless foam.

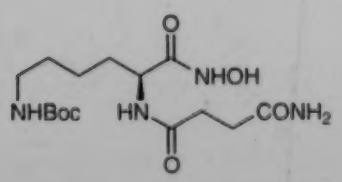
For **136ab**: IR ($CHCl_3$, cast) 3350, 3300, 1699, 1522, 1433, 1205 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(CH_3)_3$), 0.80-1.90 (m, 8H, $CH_2CH_2CH_2$, CH_2Si), 2.90 (m, 2H, CH_2SO_2), 3.73 (m, 3H, OCH_3), 3.97 (m, 1H, $CHNHSO_2$), 4.03 (m, 1H, CHNHCbz), 5.08 (s, 2H, OCH_2Ph), 5.65 (br s, 1H, NH), 5.98 (br s, 1H, NH), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) δ -2.0, 11.3, 21.9, 31.1, 32.3, 49.3, 51.7, 55.9, 66.5, 127.6, 127.8, 128.3, 137.0, 157.4, 173.3, 175.1; HRMS (ES) Calcd for $C_{21}H_{34}N_2O_8SiSNa$ 525.1703, found 525.1704, $[M+Na]^+$.

For **138ab**: IR ($CHCl_3$, cast) 3350, 3300, 1724, 1526, 1454, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(CH_3)_3$), 0.80-2.20 (m, 8H, $CH_2CH_2CH_2$, CH_2Si), 2.90 (m, 2H, CH_2SO_2), 3.72 (m, 4H, $CHNHSO_2$, OCH_3), 4.05 (m, 1H, CHNHCbz), 5.08 (s, 2H, OCH_2Ph), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 11.3, 23.0, 30.8, 32.8, 50.4, 52.8, 54.0, 57.0, 67.7, 128.9, 129.0, 129.5, 136.0, 158.3, 174.4; HRMS (ES) Calcd for $C_{21}H_{35}N_3O_8SiSNa$ 540.1812, found 540.1807, $[M+Na]^+$.



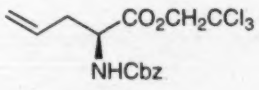
(2*S*)-1-(*N*-Benzyloxy)-2-(*N*-(3-carbomethoxylproponyl)amino)-6-((*N*-*tert*-butoxycarbonyl)amino)hexanamide (145). A solution of *N*-ε-Boc-L-lysine (0.59 mg, 2.42 mmol) in DMF (30 mL) was treated with triethylamine (0.83 mL, 6.1 mmol) followed by methyl 4-chloro-4-oxobutyrate (0.33 mL, 2.7 mmol). The solution was

allowed to stir overnight. The solvent was removed *in vacuo*, and the residue was dissolved in EtOAc (25 mL). The organic layer was washed with H_2O (3 x 10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a residue. This was immediately dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. DMAP (59 mg, 0.48 mmol), benzylhydroxylamine (0.33 g, 2.66 mmol), and EDCI hydrochloride (0.51 g, 2.66 mmol) were then added sequentially. The reaction mixture was stirred at 0 °C for 2 h, and then at rt for 15 h. The solution was washed with 1 M HCl (10 mL), followed by H_2O (2 x 10 mL). The organic layer was dried over Na_2SO_4, and the solvent was evaporated *in vacuo* to give an oil. This was purified by column chromatography (25% EtOAc in hexanes) to yield **145** as a colourless oil (0.67 g, 60%): $[\alpha]_D^{26}$ -16.4 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$, cast) 3297, 1739, 1651, 1525, 1250, 1170 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.19-1.84 (m, 15H, $CH_2CH_2CH_2$, $C(CH_3)_3$), 2.39-2.60 (m, 4H, $CH_2CH_2CO_2Me$), 3.15 (m, 1H, CH_2NHBoc), 3.62 (s, 3H, OCH_3), 4.35 (m, 1H, CHNH), 4.90 (m, 2H, $NHOCH_2Ph$), 6.90 (m, 1H, NH), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.5, 28.5, 29.2, 29.5, 30.8, 31.1, 40.1, 50.9, 51.9, 78.2, 79.3, 128.5, 128.7, 129.3, 135.3, 156.3, 169.2, 172.0, 173.5; HRMS (ES) Calcd for $C_{21}H_{35}N_3O_7Na$ 488.2373, found 488.2367, $[M+Na]^+$.



(2*S*)-1-(*N*-Hydroxy)-2-(*N*-(3-carbomethoxylproponyl)amino)-6-(*N*-*tert*-butoxycarbonyl)amino)hexanamide (146). A solution of **145** (100 mg, 0.21 mmol) in

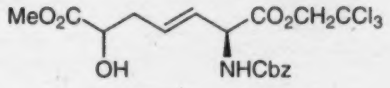
MeOH (5 mL) under argon was treated with Pd/C (10 mg). The mixture was stirred under H_2 at atmospheric pressure for 21 h and then filtered through a pad of Celite™. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography (20% NH_3 in isopropanol) to give hydroxamate **146** as a colourless foam (39 mg, 50%): $[\alpha]_D^{26}$ -1.1 (*c* 0.4, MeOH); IR ($CHCl_3$, cast) 3280, 1666, 1532 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.41 (s, 9H, $C(C\underline{H}_3)_3$), 1.19-1.84 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.39-2.60 (m, 4H, $C\underline{H}_2C\underline{H}_2CONH_2$), 3.15 (m, 1H, $C\underline{H}_2NHBoc$), 4.22 (m, 1H, $C\underline{H}NH$); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 24.1, 28.8, 30.5, 31.4, 31.9, 32.5, 41.1, 52.6, 79.9, 158.5, 171.2, 175.0, 177.4; HRMS (ES) Calcd for $C_{15}H_{28}N_4O_6Na$ 383.1907, found 383.1908, $[M+Na]^+$.



2,2,2-Trichloroethyl (2*S*)-2-(*N*-(benzyloxycarbonyl)amino)-4-pentenoate (147).

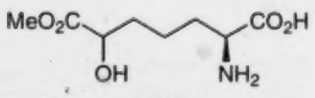
A modified procedure for the formation of the trichloroethyl ester was used for the preparation of this compound.[179] DMAP (1.55 g, 12.7 mmol) in CH_2Cl_2 (15 mL), 2,2,2-trichloroethanol (3.10 mL, 30.4 mmol) and DCC (6.30 g, 30.4 mmol) were added to a solution of acid **39** (6.3 g, 25.4 mmol) in CH_2Cl_2 (50 mL) at 0 °C under argon. The reaction mixture was stirred at rt for 18 h, filtered and concentrated *in vacuo* to give an oil, which was dissolved in EtOAc (75 mL). The solution was washed with 10% citric acid (2 x 20 mL), saturated $NaHCO_3$ (2 x 20 mL), and brine (2 x 10 mL), dried over Na_2SO_4, and concentrated to give an oil, which was purified by flash chromatography

(10% EtOAc in hexanes) to furnish **147** as a colourless oil (8.2 g, 85%): $[\alpha]_D^{26}$ -15.1 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$, cast) 3330, 1763, 1724, 1642 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.63 (m, 2H, $C\underline{H}_2CHNH$), 4.59 (dd, 1H, *J* = 13.6, 6.2 Hz, $CH_2C\underline{H}NH$), 4.66 (d, 1H, *J* = 11.9 Hz, $C\underline{H}_aH_bCCl_3$), 4.79 (d, 1H, *J* = 11.9 Hz, $CH_a\underline{H}_bCCl_3$), 5.10-5.21 (m, 4H, $C\underline{H}_2$=CH, $OC\underline{H}_2Ph$), 5.24 (br d, 1H, *J* = 7.5 Hz, N<u>H</u>), 5.71 (m, 1H, CH_2=C<u>H</u>), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.3, 53.2, 67.2, 74.5, 94.5, 120.1, 128.2, 128.3, 128.6, 131.6, 136.1, 155.8, 170.3; HRMS (ES) Calcd for $C_{16}H_{16}NO_4NaCl_3$ 402.0043, found 402.0037, $[M+Na]^+$.

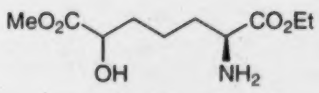


1-Methyl 7-(2,2,2-trichloroethyl) (2*RS*,6*S*)-2-hydroxy-6-(*N*-(benzyloxycarbonyl)-amino)-4-heptene-1,7-dioate (148ab). The reaction of the allylglycinate **147** (4.90 g, 12.9 mmol), methyl glyoxylate (3.4 g, 38.6 mmol), and $SnCl_4$ (15 mL, 128.7 mmol) was performed as described for the synthesis of **97ab** and gave a mixture of diastereomers **148a** and **148b** (4.0 g, 70%) as an oil: IR ($CHCl_3$, cast) 3346, 3033, 1727, 1520 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.34-2.59 (m, 2H, $C\underline{H}_2CH$=CH), 2.88 (br s, 1H, O<u>H</u>), 3.68 (s, 3H, $OC\underline{H}_3$), 4.17 (m, 1H, C<u>H</u>OH), 4.61 (d, 1H, *J* = 8.9 Hz, $C\underline{H}_aH_bCCl_3$), 4.81 (d, 1H, *J* = 8.9 Hz, $CH_a\underline{H}_bCCl_3$), 4.96 (m, 1H, C<u>H</u>NH), 5.05 (s, 2H, $OC\underline{H}_2Ph$), 5.46 (m, 1H, N<u>H</u>), 5.66-5.72 (m, 2H, C<u>H</u>=CH), 5.84-5.94 (m, 1H, CH=C<u>H</u>), 7.29-7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.0, 52.6, 55.6, 67.3, 69.8, 74.5, 94.4, 127.0, 128.2, 128.3,

128.6, 129.6, 136.0, 155.6, 169.3, 174.5; HRMS (ES) Calcd for $C_{18}H_{20}NO_7NaCl_3$ 490.0203, found 490.0196, $[M+Na]^+$.



149ab



150ab

1-Methyl (2*RS*,6*S*)-2-hydroxy-6-aminoheptane-1,7-dioic acid (149ab) and 1-Methyl 7-ethyl (2*RS*,6*S*)-2-hydroxy-6-aminoheptane-1,7-dioate (150ab).

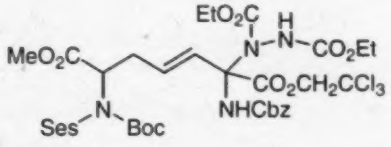
Procedure A: To a solution of **148ab** (0.10 g, 0.21 mmol) in MeOH (5 mL) was added a mixture of 10% Pd/C (10 mg) in MeOH (1 mL). The mixture was stirred under H_2 at atmospheric pressure for 10 h, filtered through a pad of Celite™ and concentrated *in vacuo* to afford an oil. This was purified by reverse phase HPLC (C_{18} Bondpak, 100% H_2O) which gave a mixture of diastereomers **149a** and **149b** (t_R 3.0 min, 5.0 mg, 12%) followed by **150a** and **150b** (35.0 mg, 70%, C_{18} Bondpak, 0-100% MeCN in H_2O over 4 min, t_R 3.8 min) as oils.

For **149ab**: IR ($CHCl_3$, cast) 3300, 1735, 1695, 1201, 1141 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.40-2.10 (m, 6H, $CH_2CH_2CH_2$), 3.85 (s, 3H, OCH_3), 4.16 (m, 1H, $CHNH_2$), 4.31 (m, 1H, CHOH); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 21.7, 31.2, 34.5, 52.5, 53.8, 70.9, 171.0, 177.7; HRMS (ES) Calcd for $C_8H_{16}NO_5$ 206.1028, found 206.1027, $[MH]^+$.

For **150ab**: IR ($CHCl_3$, cast) 3362, 1733, 1439, 1204 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.26 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.40-1.81 (m, 6H, $CH_2CH_2CH_2$), 3.85 (s, 3H, OCH_3),

3.40 (m, 1H, C<u>H</u>NH$_2$), 4.14 (m, 1H, C<u>H</u>OH), 4.18 (m, 2H, CH$_3$C<u>H</u>$_2$O); ^{13}C NMR (CD$_3$OD, 75.5 MHz) 14.4, 21.7, 31.3, 34.4, 52.5, 53.9, 63.7, 71.1, 170.5, 176.1; HRMS (ES) Calcd for $C_{10}H_{20}NO_5$ 234.1341, found 234.1341, $[MH]^+$.

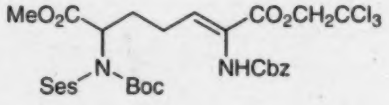
<u>Procedure B</u>: To a solution of **148ab** (0.10 g, 0.21 mmol) in MeOH (10 mL) was added 20% Pd(OH)$_2$/C (20 mg) and the mixture was hydrogenated at 30 psi for 5 h. The mixture was filtered through a pad of Celite™, concentrated *in vacuo* and purified by HPLC (as described above) to give **150ab** (37 mg, 75%) as a mixture of diastereomers. All spectral data obtained *via* this procedure are consistent with data obtained *via* procedure A.



1-Methyl 7-(2,2,2-trichloroethyl) (2*RS*,6*RS*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)amino)-6-(*N*-(benzyloxycarbonyl)amino))-6-(*N*,*N*′-diethoxycarbonylhydrazino)-4-heptene-1,7-dioate (151a-d).

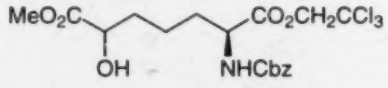
To a solution of triphenylphosphine (0.14 g, 0.53 mmol) and **103** (0.14 g, 0.49 mmol) in THF (6 mL) at 0 °C was added **148ab** (0.15 g, 0.32 mmol) in THF (2 mL). To the resulting mixture was added DEAD (77 µL, 0.49 mmol) dropwise over a 5 min period. The solution was stirred for 4 h and concentrated *in vacuo*. The residue was purified by HPLC (C_{18} Bondpak, gradient elution: 25-80% MeCN in H$_2$O over 25 min, t_R 19.9 min) to give an inseparable mixture of four diastereomers **151a-d** as a colourless oil (0.14 g, 50%): IR ($CHCl_3$, cast) 3377, 1725, 1495, 1252, 1139 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ

0.05 (s, 9H, Si(C<u>H</u>$_3$)$_3$), 1.02-1.30 (m, 8H, C<u>H</u>$_2$Si, 2 x C<u>H</u>$_3$CH$_2$O), 1.47 (s, 9H, C(C<u>H</u>$_3$)$_3$), 2.66-3.06 (m, 2H, C<u>H</u>$_2$CH=CH), 3.47 (m, 2H, C<u>H</u>$_2$SO$_2$), 3.71 (s, 3H, OC<u>H</u>$_3$), 4.17 (m, 4H, 2 x CH$_3$C<u>H</u>$_2$O), 4.70 (m, 2H, C<u>H</u>$_2$CCl$_3$), 4.85 (m, 1H, C<u>H</u>N), 5.08 (s, 2H, OC<u>H</u>$_2$Ph), 5.70 (m, 2H, C<u>H</u>=CH), 6.00 (m, 1H, CH=C<u>H</u>), 6.80 (br s, 1H, N<u>H</u>), 7.26-7.38 (m, 5H, Ph), 7.60 (br s, 1H, N<u>H</u>); ^{13}C NMR ($CDCl_3$, 125 MHz) δ -2.2, 9.7, 14.4, 14.5, 27.9, 33.5, 50.9, 52.7, 58.6, 61.7, 63.2, 67.1, 75.7, 76.3, 85.2, 94.2, 126.1, 127.9, 128.0, 128.2, 128.6, 132.0, 136.0, 150.8, 154.7, 155.2, 155.6, 166.4, 169.7; HRMS (ES) Calcd for $C_{34}H_{51}N_4O_{14}SiSCl_3Na$ 927.1855, found 927.1895, $[M+Na]^+$.



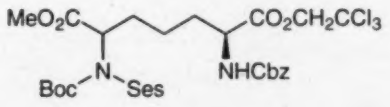
1-Methyl 7-(2,2,2-trichloroethyl) (2*RS*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)amino))-6-(*N*-(benzyloxycarbonyl)amino)-5-heptene-1,7-dioate (152ab). To a solution of triphenylphospine (93.0 mg, 0.36 mmol) in THF (5 mL) at –78 °C was added DEAD (49 μL, 0.33 mmol) dropwise over 5 min. The solution was stirred for 30 min and treated with a solution of **148ab** (127 mg, 0.27 mmol) and carbamate **103** (92 mg, 0.33 mmol) in THF (2 mL) dropwise over 1 min. The solution was allowed to warm to rt over several hours. The solvent was evaporated *in vacuo*. The resultant residue was purified by reverse phase HPLC (C_{18} Bondpak, 25-69% MeCN in H_2O over 22 min) to give a mixture of diastereomers **152a** and **152b** (t_R 19.3 min, 25 mg, 13%), a mixture of diastereomers **151a-d** (t_R 13.3 min, 20 mg, 8%) and recovered starting

material **148ab** (t_R 12.1 min, 30 mg, 16%) as oils. For **152ab**: IR ($CHCl_3$, cast) 3353, 1731, 1652, 1560, 1499, 1251, 1138 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 1.00-1.30 (m, 2H, $C\underline{H}_2Si$), 1.49 (s, 9H, $C(C\underline{H}_3)_3$), 2.10-2.50 (m, 4H, $C\underline{H}_2C\underline{H}_2$), 3.50 (m, 2H, $C\underline{H}_2SO_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 4.75 (m, 3H, $C\underline{H}_2CCl_3$, C<u>H</u>N), 5.08 (s, 2H, $OC\underline{H}_2Ph$), 6.30 (br s, 1H, N<u>H</u>), 6.78 (m, 1H, C<u>H</u>=C), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.7, 14.2, 21.1, 25.5, 28.0, 29.1, 50.9, 52.6, 58.9, 60.4, 67.5, 74.7, 85.3, 94.7, 125.9, 128.0, 128.2, 128.2, 128.6, 128.8, 136.1, 138.8, 150.9, 154.2, 162.9, 170.2; HRMS (ES) Calcd for $C_{28}H_{41}N_2O_{10}SiSCl_3Na$ 753.1214, found 753.1206, $[M+Na]^+$.

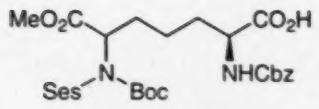


1-Methyl 7-(2,2,2-trichloroethyl)-(2*RS*,6*S*)-2-hydroxy-6-(*N*-(benzyloxycarbonyl)-amino)heptane-1,7-dioate (159ab). A solution of dehydropimelate **148ab** (0.74 g, 1.57 mmol) in EtOAc (5 mL) was treated with 5% Rh/C (200 mg) and agitated under H_2 at atmospheric pressure for 3 days. The mixture was filtered through a pad of Celite™, and concentrated *in vacuo* to afford a mixture of diastereomers **159a** and **159b** (0.45 g, 60%) as a colourless oil: IR ($CHCl_3$, cast) 3346, 1727, 1526, 1454, 1248 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.41-2.00 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.85 (m, 1H, O<u>H</u>), 3.85 (s, 3H, $OC\underline{H}_3$), 4.16 (m, 1H, C<u>H</u>OH), 4.51 (m, 1H, C<u>H</u>NH), 4.63 (d, 1H, J = 9.0 Hz, $C\underline{H}_aH_bCCl_3$), 4.88 (d, 1H, J = 9.0 Hz, $CH_a\underline{H}_bCCl_3$), 5.11 (s, 2H, $OC\underline{H}_2Ph$), 5.36 (d, 1H, J = 6.1 Hz, N<u>H</u>), 7.27-7.33 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.7, 31.9, 33.5, 52.6, 53.7, 67.2, 69.9, 74.3,

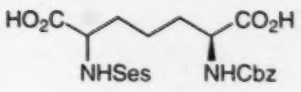
94.5, 128.2, 128.3, 128.6, 136.1, 155.9, 170.9, 175.3; HRMS (ES) Calcd for $C_{18}H_{22}NO_7NaCl_3$ 492.0360, found 492.0357, $[M+Na]^+$; Anal. Calcd for $C_{18}H_{22}NO_7Cl_3$: C, 45.93; H, 4.71; N, 2.98. Found: C, 45.66; H, 4.68; N, 2.86.



1-Methyl 7-(2,2,2-trichloroethyl) (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)amino))-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (160ab). Reaction of **159ab** (2.05 g, 4.27 mmol) with triphenylphosphine (1.70 g, 6.83 mmol), carbamate **103** (1.50 g, 5.33 mmol) and DEAD (1.0 mL, 6.40 mmol) was performed as described for compound **104ab** and gave an oil, that was purified by flash chromatography (20% EtOAc in hexanes) to furnish a mixture of diastereomers **160a** and **160b** (2.25 g, 70%) as a colourless oil: IR ($CHCl_3$, cast) 3373, 1728, 1520, 1251, 1138 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 1.00-2.20 (m, 17H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2Si$, $C(C\underline{H}_3)_3$), 3.40-3.61 (m, 2H, $C\underline{H}_2SO_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 4.50 (m, 1H, C<u>H</u>NH), 4.60-4.94 (m, 3H, C<u>H</u>N, $OC\underline{H}_2CCl_3$), 5.08 (s, 2H, $OC\underline{H}_2Ph$), 5.30 (m, 1H, N<u>H</u>), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.7, 22.1, 28.0, 28.9, 31.7, 50.8, 52.5, 53.7, 58.7, 67.1, 74.3, 85.2, 94.6, 128.1, 128.5, 136.3, 150.9, 156.1, 170.4, 170.9; HRMS (ES) Calcd for $C_{28}H_{43}N_2O_{10}SiSCl_3Na$ 755.1371, found 755.1375, $[M+Na]^+$; Anal. Calcd for $C_{28}H_{43}N_2O_{10}SiSCl_3$: C, 45.81; H, 5.90; N, 3.82. Found: C, 45.47; H, 5.92; N, 3.71.



161ab



162ab

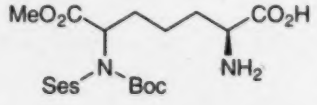
Methyl (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)-amino)-6-(*N*-(benzyloxycarbonyl)amino))heptane-7-oic acid (161ab) and (2*RS*,6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(*N*-(2-trimethylsilylethylsulfonyl)-amino)heptane-1,7-dioic acid (162ab).

Procedure A: A modified procedure was used[183] for the removal of the trichloroethyl ester moiety. To a 0.1 M solution of samarium iodide (92 mL, 7.41 mmol) cooled to 0 °C under argon was added **160ab** (1.35 g, 1.84 mmol) dropwise over 2 min. The resultant solution was stirred for 3 h and then quenched by the careful addition of 6 M HCl. The yellow solution was diluted with EtOAc (20 mL), and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 x 50 mL), and the combined organic layers were dried over Na_2SO_4, and evaporated *in vacuo* to afford a yellow oil. This was purified by flash chromatography (60% EtOAc in hexanes) to furnish a mixture of diastereomers **161a** and **161b** as a white foam (0.95 g, 86%): IR ($CHCl_3$, cast) 3373, 1728, 1520, 1455, 1251, 1138 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(CH_3)_3$), 1.00-2.20 (m, 17H, $CH_2CH_2CH_2$, CH_2Si, $C(CH_3)_3$), 3.40-3.61 (m, 2H, CH_2SO_2), 3.71 (s, 3H, OCH_3), 4.37 (m, 1H, CHNH), 4.74 (m, 1H, CHN), 5.10 (s, 2H, OCH_2Ph), 5.46 (m, 1H, NH), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.6, 22.0, 27.9,

29.8, 31.7, 50.8, 52.5, 53.6, 58.8, 67.0, 85.2, 128.0, 128.5, 136.3, 150.9, 156.3, 170.5, 176.7; HRMS (ES) Calcd for $C_{26}H_{42}N_2O_{10}SiSNa$ 625.2227, found 625.2219, $[M+Na]^+$.

Procedure B: The above procedure for the reaction of **160ab** (0.47 g, 0.64 mmol) with a 0.1 M solution of samarium iodide (44 mL, 4.48 mmol) was followed with the exception that the reaction mixture was stirred for *6 h* at rt to furnish **161a** and **161b** as a white foam (0.32 g, 86%), a diastereomeric mixture of **162a** and **162b** (16 mg, 5%) as a white foam, as well as compound **136ab** (5%).

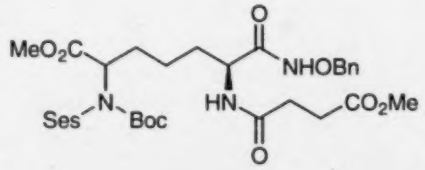
For **162ab**: IR (MeOH, cast) 3373, 1728, 1520, 1455 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 0.05 (s, 9H, $Si(CH_3)_3$), 1.00-2.20 (m, 8H, $CH_2CH_2CH_2$, CH_2Si), 2.94 (m, 2H, CH_2SO_2), 3.94 (m, 1H, $CHNHSO_2$), 4.14 (m, 1H, CHNHCbz), 5.10 (s, 2H, OCH_2Ph), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.1, 11.3, 23.0, 32.2, 33.6, 50.3, 55.2, 57.2, 67.6, 128.5, 128.8, 128.9, 138.2, 158.7, 175.6, 175.9; HRMS (ES) Calcd for $C_{20}H_{32}N_2O_8SiSNa$ 511.1541, found 511.1544, $[M+Na]^+$. Spectroscopic data obtained for **136ab** were consistent with previously reported data.



Methyl (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)-amino))-6-aminoheptane-7-oic acid (163ab).

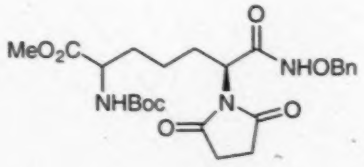
To a solution of **161ab** (0.90 g, 1.50 mmol) in EtOAc: isopropanol (8:2, 20 mL) was added 10% Pd/C (100 mg). The mixture was stirred under H_2 at atmospheric pressure for

21 h, then filtered through Celite™, and the solvent was removed *in vacuo* to afford a mixture of diastereomers **163a** and **163b** (625 mg, 90%) as a white solid: IR ($CHCl_3$, cast) 2952, 1730, 1632, 1511, 1252, 1140 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 1.00-2.20 (m, 17H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2Si$, 9H, $C(C\underline{H}_3)_3$), 3.48-3.70 (m, 3H, $C\underline{H}NH$, $C\underline{H}_2SO_2$), 3.72 (s, 3H, $OC\underline{H}_3$), 4.76 (m, 1H, $C\underline{H}N$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.3, 9.7, 15.3, 23.0, 27.9, 30.5, 50.7, 52.5, 54.8, 65.9, 85.1, 150.9, 170.6, 174.1; HRMS (ES) Calcd for $C_{18}H_{36}N_2O_8SiSNa$ 491.1859, found 491.1855, $[M+Na]^+$.



1-Methyl (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)-amino))-6-(*N*-(3-carbomethoxylproponyl)amino)heptane-1,7-dioate, 7-*N*-benzyloxyamide (164ab). A solution of **163ab** (0.63 g, 1.34 mmol) in CH_2Cl_2 (30 mL) was treated with triethylamine (0.42 mL, 2.95 mmol) followed by methyl-4-chloro-4-oxobutyrate (0.36 mL, 2.95 mmol). The solution was stirred overnight. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc (25 mL). The organic layer was washed with H_2O (3 x 10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a crude residue. This was immediately dissolved in THF (10 mL) and cooled to 0 °C, treated sequentially with triethylamine (224 μL, 1.61 mmol) and ethyl chloroformate (141 μL, 1.47 mmol) and stirred for 1 h. Triethylamine (374 μL, 2.68 mmol) and benzylhydroxylamine hydrochloride (0.43 g, 2.68 mmol) were added to the reaction

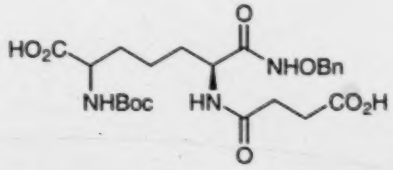
mixture which was then stirred for 15 h at rt. The solution was washed with 1 M HCl (10 mL) followed by water (2 x 10 mL). The organic layer was dried over Na_2SO_4, and the solvent was evaporated *in vacuo* to give an oil. This was purified by column chromatography (25% EtOAc in hexanes) to yield a mixture of diastereomers **164a** and **164b** (600 mg, 65% overall) as a colourless oil: IR ($CHCl_3$, cast) 3213, 2952, 1713, 1644, 1538, 1251, 1169 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 1.00-2.22 (m, 8H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2Si$), 1.47 (s, 9H, $C(C\underline{H}_3)_3$), 2.40-2.80 (m, 4H, $C\underline{H}_2C\underline{H}_2CO_2Me$), 3.48-3.70 (m, 2H, $C\underline{H}_2SO_2$), 3.63 (s, 3H, $OC\underline{H}_3$), 3.72 (s, 3H, $OC\underline{H}_3$), 4.25 (m, 1H, C<u>H</u>NH), 4.73 (m, 1H, C<u>H</u>N), 4.84 (d, 1H, J = 12.0 Hz, $NHOC\underline{H}_aH_bPh$), 4.88 (d, 1H, J = 12 Hz, $NHOCH_a\underline{H}_bPh$), 7.26-7.38 (m, 5H, Ph), 6.29 (m, 1H, N<u>H</u>), 6.39 (m, 1H, N<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.7, 21.6, 27.8, 28.8, 29.3, 30.7, 30.8, 50.7, 51.9, 52.6, 58.4, 58.9, 78.2, 85.4, 128.5, 128.6, 129.3, 129.3, 135.3, 150.7, 170.4, 172.1, 172.6, 173.7; HRMS (ES) Calcd for $C_{30}H_{49}N_3O_{11}SiSNa$ 710.2755, found 710.2749, $[M+Na]^+$.



1-Methyl (2*RS*,6*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-6-(*N*-(cyclopentane-1,3-dione) heptane-1,7-dioate, 7-*N*-benzyloxyamide (165ab).

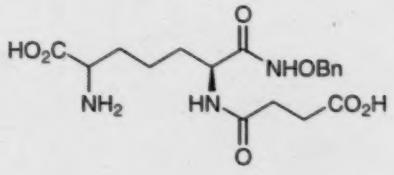
To a solution of **164ab** (73.0 mg, 0.11 mmol) in THF (3 mL) was added 1.0 M TBAF in THF (300 μL, 0.32 mmol). The solution was stirred at rt for 1 h, and the solvent was

evaporated *in vacuo*. The residue was purified by flash chromatography (50% EtOAc in hexanes) to furnish a mixture of diastereomers **165a** and **165b** (45.9 mg, 90%) as a colourless oil: IR ($CHCl_3$, cast) 3332, 2954, 1707, 1504, 1455, 1252, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.00-1.90 (m, 15H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 2.69 (s, 4H, $NH(CO)_2C\underline{H}_2C\underline{H}_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 4.20 (m, 1H, C<u>H</u>NHBoc), 4.60 (m, 1H, C<u>H</u>N), 4.86 (s, 1H, $NHOC\underline{H}_2Ph$), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.2, 22.2, 25.3, 27.2, 28.0, 28.4, 32.3, 52.4, 53.7, 78.1, 80.3, 128.6, 128.6, 128.9, 129.4, 135.1, 155.5, 160.6, 166.1, 172.9, 177.0 ; HRMS (ES) Calcd for $C_{23}H_{33}N_3O_8Na$ 514.2165, found 514.2165, $[M+Na]^+$.



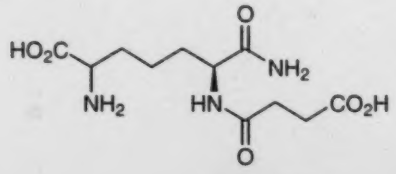
(2*RS*, 6*S*)-2-(*N*-(*tert*-Butoxycarbonyl)amino)-6-(*N*-(3-carboxyproponyl)amino) heptane-1,7-dioic acid, 7-*N*-benzyloxyamide (166ab). A solution of **165ab** (39 mg, 80 μmol) in a THF:H_2O mixture (1:1, 1 mL) was treated with lithium hydroxide monohydrate (13 mg, 0.12 mmol). The resulting solution was stirred for 18 h at rt and the solvent was removed *in vacuo* to yield a residue. This was redissolved in H_2O (5 mL). The aqueous solution was washed with EtOAc (10 mL), acidified to pH 2 using 6 M HCl, and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over Na_2SO_4, and concentrated *in vacuo* to give a crude residue. This was purified by reverse phase HPLC (C_{18} Bondpak, gradient elution: 0-60% MeCN in H_2O over 10 min) to give a

mixture of diastereomers **166a** and **166b** (t_R 9.1 min, 35 mg, 90%) as an oil: IR (MeOH, cast) 3319, 3288, 2954, 1732, 1716, 1695, 1685, 1665, 1575, 1546 cm^{-1}; ^1H NMR (CD_3CN, 300 MHz) δ 1.00-1.90 (m, 15H, $CH_2CH_2CH_2$, $C(CH_3)_3$), 2.40-2.58 (m, 4H, $CH_2CH_2CO_2H$), 4.02 (m, 1H, CHNHBoc), 4.16 (m, 1H, CHNH), 4.80 (s, 2H, $NHOCH_2Ph$), 5.69 (br s, 1H, NH), 6.97 (br s, 1H, NH), 7.26-7.35 (m, 5H, Ph), 9.80 (br s, 1H, NH); ^{13}C NMR (CD_3CN, 125 MHz) δ 22.5, 28.4, 29.9, 30.4, 31.0, 31.8, 32.0, 32.1, 32.4, 51.8, 55.2, 78.6, 80.1, 129.4, 129.5, 130.2, 136.6, 156.9, 169.9, 173.6, 174.9, 175.1; HRMS (ES) Calcd for $C_{23}H_{33}N_3O_9Na$ 518.2115, found 518.2124, $[M+Na]^+$.

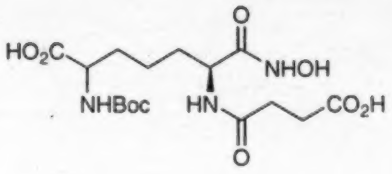


(2*RS*,6*S*)-2-Amino-6-(*N*-(3-carboxyproponyl)amino)heptane-1,7-dioic acid, 7-*N*-benzyloxyamide (167ab). A solution of **166ab** (24.0 mg, 0.05 mmol) in CH_2Cl_2 (200 μL) was treated with TFA (50 μL, 0.60 mmol). An additional portion of CH_2Cl_2 (300 μL) was added, and the solution was stirred for 2 h. Removal of the CH_2Cl_2 and TFA *in vacuo* afforded a crude solid which was purified by flash chromatography (30% NH_3 in isopropanol) to afford a mixture of diastereomers **167a** and **167b** (18 mg, 91%) as an oil: IR (μscope) 3310, 3288, 2957, 1645, 1547 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.20-1.90 (m, 6H, $CH_2CH_2CH_2$), 2.50 (m, 4H, $CH_2CH_2CO_2H$), 3.66 (m, 1H, $CHNH_2$), 4.11 (m, 1H, CHNH), 4.87 (s, 2H, $NHOCH_2Ph$), 7.43 (m, 5H, Ph); ^{13}C NMR (D_2O, 75.5 MHz) δ 21.6,

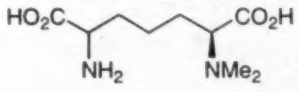
30.6, 30.7, 31.2, 31.8, 52.2, 55.3, 79.0, 129.5, 130.1, 130.9, 135.2, 171.0, 175.3, 176.3, 179.8; HRMS (ES) Calcd for $C_{18}H_{26}N_3O_7$ 396.1765, found 396.1767, $[MH]^+$.



(*2RS,6S*)-2-Amino-6-(*N*-(3-carboxyproponyl)amino)-7-amide heptanoic acid (168ab). To a solution of **167ab** (10.0 mg, 0.03 mmol) in H_2O (800 µL) and MeOH (200 µL) was added 10% Pd/C (2.5 mg). The mixture was stirred under H_2 at atmospheric pressure for 3 h. The catalyst was removed by filtration through Celite™, and the solvent was evaporated *in vacuo* to give a solid. This was purified by preparative TLC on silica gel (30% NH_3 in isopropanol) to give a mixture of diastereomers **168a** and **168b** (5 mg, 68%) as a solid: IR (µscope) 3310, 3186, 2957, 1640, 1559 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.40-1.90 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.50 (m, 4H, $C\underline{H}_2C\underline{H}_2CO_2H$), 3.73 (dd, 1H, J = 12.3, 6.3 Hz, $C\underline{H}NH_2$), 4.26 (dd, 1H, J = 9.5, 4.5 Hz, $C\underline{H}NH$); ^{13}C NMR (D_2O, 125 MHz) δ 21.1, 30.1, 30.6, 31.9, 32.4, 53.5, 54.8, 174.8, 176.5, 177.3, 180.7; HRMS (ES) Calcd for $C_{11}H_{20}N_3O_6$ 290.1347, found 290.1346, $[MH]^+$.

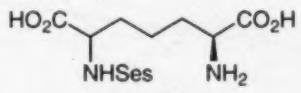


(2*RS*,6*S*)-2-(*N*-(*tert*-Butoxycarbonyl)amino)-6-(*N*-(3-carboxyproponyl)amino) heptane-1,7-dioic acid, 7-*N*-hydroxyamide (169ab). A mixture of **166ab** (6.0 mg, 0.012 mmol) and 10% Pd/C (1.0 mg) in isopropanol (0.5 mL) was stirred under H_2 at atmospheric pressure for 4 h. The mixture was filtered through Celite™, and the solvent was removed *in vacuo* to afford a mixture of diastereomers **169a** and **169b** (3.0 mg, 61%) as a white solid: ^{1}H NMR (CD_3OD, 300 MHz) δ 1.20-1.90 (m, 15H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 2.40-2.62 (m, 4H, $C\underline{H}_2C\underline{H}_2CO_2H$), 4.01 (m, 1H, $C\underline{H}NH_2$), 4.24 (m, 1H, $C\underline{H}NH$); ^{13}C NMR (CD_3OD, 125 MHz) δ 23.3, 25.2, 28.7, 30.3, 31.5, 32.7, 52.4, 55.1, 80.5, 171.2, 174.7, 176.6; HRMS (ES) Calcd for $C_{16}H_{27}N_3O_9Na$ 428.1645 found 428.1640, $[M+Na]^+$.



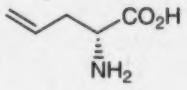
(2*RS*,6*S*)-2-Amino-6-(*N*,*N*´-dimethylamino)heptane-1,7-dioic acid (173ab). Compound **187ab** (21 mg, 61 μmol) was reacted with lithium hydroxide monohydrate (5.8 mg, 0.14 mmol) in MeCN/H_2O (1:1, 800 μL) for 18 h. The solvent was removed *in vacuo*, and the residue was dissolved in H_2O (5 mL). This solution was washed with EtOAc (2 x 5 mL). The aqueous layer was acidified to pH 2 with 6 M HCl, and allowed

to stand at rt for 2 h. The H_2O was removed *in vacuo* and the residue was purified by flash chromatography (30% ammonia in isopropanol) to give a mixture of diastereomers **173a** and **173b** (6.0 mg, 46%) as a white solid: IR (μscope) 3500-2900, 1606, 1529, 1463, 1360 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.20-2.20 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.98 (s, 6H, $N(C\underline{H}_3)_2$), 3.85 (m, 1H, $C\underline{H}N(CH_3)_2$), 4.09 (m, 1H, $C\underline{H}NH_2$); ^{13}C NMR (D_2O, 75.5 MHz) δ 21.5, 27.4, 30.3, 41.0, 43.8, 53.6, 69.7, 172.3, 172.9; HRMS (ES) Calcd for $C_9H_{19}N_2O_4$ 219.1339, found 219.1340, $[MH]^+$.

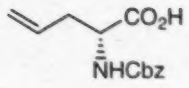


(2*RS*,6*S*)-2-(*N*-(2-Trimethylsilylethylsulfonyl)amino)-6-aminoheptane-1,7-dioic acid (175ab). To a solution of **104ab** (0.18 g, 0.29 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.22 mL, 2.9 mmol) and the solution was stirred at rt for 3 h. The solvent was evaporated, and the resulting residue was dried *in vacuo* overnight. The residue was dissolved in MeCN:H_2O (1:1, 2 mL), reacted with lithium hydroxide monohydride (48 mg, 1.2 mmol) and stirred overnight. The solvent was evaporated *in vacuo* and the residue was redissolved in H_2O (5 mL) and acidified to pH 2 using conc. HCl. The products were extracted with EtOAc (3 x 10 mL) and the solvent was evaporated. The crude product was dissolved in MeOH (5 mL) containing 10% Pd/C (20 mg) and stirred under H_2 at atmospheric pressure for 21 h. The reaction mixture was filtered through a pad of Celite™ and MeOH was removed *in vacuo* to give an oil. Purification of this oil by reverse-phase HPLC (C_{18} Bondpak, gradient elution, 0-12% MeCN in H_2O over 15

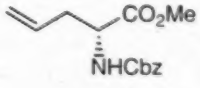
min) afforded a mixture of diastereomers **175a** and **175b** (t_R 14.0 min, 70 mg, 70%) as a white solid: IR (μscope) 3154, 2954, 1723, 1624, 1512, 1348, 1251cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.05 (s, 9H, $Si(C\underline{H}_3)_3$), 1.00 (m, 2H, $C\underline{H}_2Si$), 1.50-2.00 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.96 (m, 2H, $C\underline{H}_2SO_2$), 3.66 (dd, 1H, J = 12.3, 6.7 Hz, $C\underline{H}NH_2$), 3.94 (dd, 1H, J = 8.3, 4.9 Hz, $C\underline{H}NHSO_2$); ^{13}C NMR (CD_3OD, 75.5 MHz) δ -2.0, 11.2, 22.6, 31.5, 33.9, 50.2, 55.3, 57.4, 173.9, 175.9; HRMS (ES) Calcd for $C_{12}H_{27}N_2O_6SiS$ 355.1359, found 355.1362, $[MH]^+$.



(2*R*)-2-Amino-4-pentenoic acid (177). The procedure of Black and Wright[97] was adapted to synthesize this known compound. To a solution of **35** (30.1 g, 0.19 mol) in H_2O (100 mL) was added 2 M HCl (423 mL). This acidic solution was then heated to reflux for 3 h. The water was removed *in vacuo* and the product was freeze dried overnight. The resulting white solid was recrystallized from H_2O/EtOH to give **177** (15 g, 69%) as white crystals: mp 154-156 °C (lit[97] mp 158-159 °C); $[\alpha]_D^{26}$ +36.5 (*c* 4.0, H_2O) (lit[97] $[\alpha]_D$ +37.1 (*c* 4.0, H_2O)); IR (Nujol) 3210, 1720, 1642 cm^{-1}; ^{1}H NMR (D_2O, 300 MHz) δ 2.55-2.74 (m, 2H, $C\underline{H}_2CHNH$), 4.10 (dd, 1H, J =7.0, 5.2 Hz, $C\underline{H}NH$)), 5.25 (m, 2H, $C\underline{H}_2$=CH), 5.75 (m, 1H, CH_2=$C\underline{H}$); ^{13}C NMR (D_2O, 75.5 MHz) δ 34.8, 53.1, 122.3, 131.1, 172.3; HRMS (EI) Calcd for $C_5H_9NO_2$ 115.0633, found 115.0636, $[M]^+$.

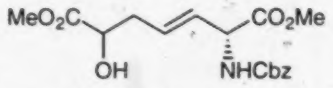


(2*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-pentenoic acid (178). The procedure to make **39** was employed to convert **177** (5.0 g, 43.4 mmol) to **178** (8.10 g, 74%): $[\alpha]_D^{26}$ -17.3 (*c* 1.0, $CHCl_3$) (lit[97] $[\alpha]_D^{26}$ -13.0 (*c* 5.0, $CHCl_3$)); IR ($CHCl_3$, cast) 3316, 1718, 1520, 1455, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.48-2.70 (m, 2H, C<u>H</u>$_2$CHNH), 4.47 (dd, 1H, *J* =13.5, 6.5 Hz, C<u>H</u>NH), 5.10 (m, 4H, CH=C<u>H</u>$_2$, C<u>H</u>$_2$Ph), 5.40 (d, 1H, *J* = 7.8 Hz, N<u>H</u>), 5.61-5.78 (m, 1H, C<u>H</u>=CH$_2$), 7.25-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.4, 53.2, 67.3, 119.7, 128.1, 128.3, 128.5, 131.8, 136.1, 156.1, 176.3; HRMS (ES) Calcd for $C_{13}H_{15}NO_4Na$ 272.0899, found 272.0900, $[M+Na]^+$; Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.56; H, 6.03; N, 5.55.

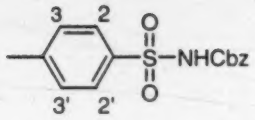


Methyl (2*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-pentenoate (179). Compound **178** (7.11 g, 28.50 mmol) was reacted with freshly prepared diazomethane[209] as described for **34** to furnish **179** (7.00 g, 85%) as a colourless oil: $[\alpha]_D^{26}$ -16.3 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$, cast) 3340, 1723, 1642, 1525, 1454, 1216, 739, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.53 (m, 2H, C<u>H</u>$_2$CHNH), 3.60 (s, 3H, OC<u>H</u>$_3$), 4.40 (dd, 1H, *J* = 7.4, 6.1 Hz, C<u>H</u>NH), 5.07-5.18 (m, 4H, C<u>H</u>$_2$=CH, C<u>H</u>$_2$Ph), 5.67 (m, 2H, CH$_2$=C<u>H</u>, N<u>H</u>), 7.25-7.32 (m, 5H, Ph);

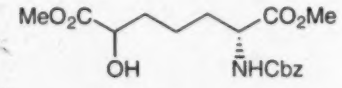
^{13}C NMR ($CDCl_3$, 150 MHz) δ 36.2, 51.8, 53.2, 66.5, 118.6, 127.7, 127.7, 128.1, 132.0, 136.2, 155.5, 171.8; HRMS (ES) Calcd for $C_{14}H_{17}NO_4Na$ 286.1055, found 286.1057, $[M+Na]^+$; Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.59; H, 6.56; N, 5.34.



Dimethyl (2*RS*,6*R*)-2-hydroxy-6-(*N*-(benzyloxycarbonyl)amino)-4-heptene-1,7-dioate (180ab). The procedure to make **97ab** was employed to react **179** (5.0 g, 19.0 mmol), methyl glyoxylate (5.0 g, 57.0 mmol), and $SnCl_4$ (22 mL, 189.0 mmol) to furnish a mixture of diastereomers **180a** and **180b** (4.75 g, 71%) as an oil: IR ($CHCl_3$, cast) 3357, 3032, 2953, 1742, 1521, 1454, 1214 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.34-2.59 (m, 2H, C<u>H</u>$_2$CH=CH$_2$), 3.02 (br s, 1H, OH), 3.60-3.78 (m, 6H, 2 x OC<u>H</u>$_3$), 4.20-4.26 (m, 1H, C<u>H</u>OH), 4.80-4.89 (m, 1H C<u>H</u>NH), 5.10 (s, 2H, C<u>H</u>$_2$Ph), 5.60-5.65 (m, 2H, C<u>H</u>=CH), 5.72-5.82 (m, 1H, CH=C<u>H</u>), 7.29-7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.9, 52.4, 52.6, 55.5, 67.0, 69.8, 128.0, 128.1, 128.5, 128.6, 136.2, 155.5, 171.1, 174.4; HRMS (ES) Calcd for $C_{17}H_{21}NO_7Na$ 374.1216, found 374.1218, $[M+Na]^+$; Anal. Calcd for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02; N, 3.99. Found: C, 57.74; H, 6.04; N, 3.85.

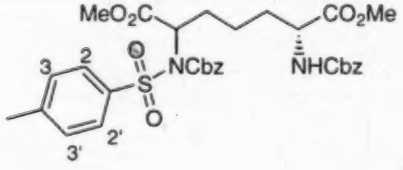


***N*-(Benzyloxycarbonyl)-4-methylbenzenesulfonamide (182).** A modified literature procedure was used.[188] To a solution of *p*-toluenesulfonamide (5.00 g, 29.21 mmol) in CH_2Cl_2 (70 mL) was added triethylamine (5.29 mL, 37.90 mmol), DMAP (0.26 g, 2.92 mmol) and benzyl chloroformate (4.40 mL, 30.60 mmol). The solution was stirred for 4 h, and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (50% EtOAc in hexanes) to give the sulfonamide **182** (5.35 g, 60%) as a white solid: IR ($CHCl_3$ cast) 3203, 2953, 1721, 1663, 1596, 1540, 1495, 1320, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.41 (s, 3H, C<u>H</u>$_3$), 5.07 (s, 2H, OC<u>H</u>$_2$Ph), 7.30 (m, 7H, CH_2<u>Ph</u>, and 3-C<u>H</u>, 3′-C<u>H</u>), 7.86 (m, 2H, 2-C<u>H</u>, 2′-C<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.7, 68.6, 128.4, 128.6, 128.7, 129.6, 134.4, 135.4, 145.0, 150.5; HRMS (ES) Calcd for $C_{15}H_{15}NO_4SNa$ 328.0620, found 328.0622, $[M+Na]^+$; Anal. Calcd for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59. Found: C, 58.76; H, 4.94; N, 4.54.



Dimethyl (2*RS*,6*R*)-2-hydroxy-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (183ab). The procedure for the synthesis of **100ab** was used to transform **180ab** (2.87 g,

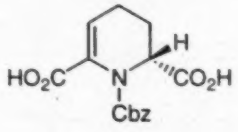
8.17 mmol) into a mixture of diastereomers **183a** and **183b** (2.0 g, 70%) as a colourless oil: IR ($CHCl_3$, cast) 3354, 2953, 1735, 1529, 1455, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40-1.95 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.95 (br s, 1H, O<u>H</u>), 3.75 (s, 3H, $OC\underline{H}_3$), 3.76 (s, 3H, $OC\underline{H}_3$), 4.16 (m, 1H, C<u>H</u>OH), 4.35 (dd, 1H, *J* = 13.8, 9.0 Hz, C<u>H</u>NH), 5.10 (s, 2H, $OC\underline{H}_2Ph$), 5.36 (d, 1H, *J* = 8.6 Hz, N<u>H</u>), 7.28-7.36 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.6, 32.2, 33.6, 52.4, 53.6, 67.0, 70.1, 128.1, 128.2, 128.5, 136.2, 155.9, 172.8, 175.3; HRMS (ES) Calcd for $C_{17}H_{23}NO_7Na$ 376.1372, found 376.1375, $[M+Na]^+$.



Dimethyl (2*RS*,6*R*)-2-((*N*-(benzyloxycarbonyl)-*N*-(*p*-toluenesulfonyl)amino))-6-(*N*-(benzyloxycarbonyl)amino)heptane-1,7-dioate (184ab).

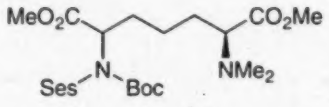
Reaction of **183ab** (0.79 g, 2.23 mmol) with triphenylphosphine (0.96 g, 3.66 mmol), sulfonamide **182** (0.85 g, 2.79 mmol) and DEAD (0.54 mL, 3.42 mmol) was performed as described for compound **104ab** and gave an oil. This was purified by flash chromatography (20% EtOAc in hexanes) to furnish a mixture of diastereomers **184ab** (0.93 g, 65%) as a colourless oil: IR ($CHCl_3$, cast) 3360, 2952, 1735, 1596, 1520, 1455, 1218 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40-1.95 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 3.59 (s, 3H, $OC\underline{H}_3$), 3.73 (s, 3H, $OC\underline{H}_3$), 4.38 (m, 1H, C<u>H</u>NH), 5.00-5.15 (m, 5H, 2 x $OC\underline{H}_2Ph$, C<u>H</u>N), 5.40 (d, 1H, *J* = 8.6 Hz, N<u>H</u>), 7.05-7.40 (m, 12H, 2 x $OCH_2\underline{Ph}$, and 3-C<u>H</u>, 3′-C<u>H</u>), 7.86

(m, 2H, 2-C<u>H</u>, 2′-C<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.6, 22.1, 29.3, 31.8, 52.3, 53.6, 59.3, 66.9, 69.1, 128.0, 128.4, 128.5, 128.9, 129.0, 134.2, 135.7, 136.3, 144.6, 151.3, 155.9, 169.9, 172.8; HRMS (ES) Calcd for $C_{32}H_{36}N_2O_{10}SNa$ 663.1988, found 663.1993, $[M+Na]^+$; Anal. Calcd for $C_{32}H_{36}N_2O_{10}S$: C, 59.98; H, 5.66; N, 4.37. Found: C, 59.58; H, 5.59; N, 4.33.

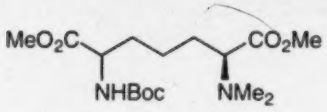


(2*R*)-*N*-(Benzyloxycarbonyl)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylate (185). To a solution of **184ab** (0.50 g, 0.78 mmol) in MeCN:H_2O (1:1, 10 mL) was added lithium hydroxide monohydrate (0.098 g, 2.34 mmol). The mixture was stirred at rt for 8 h. The solvent was removed *in vacuo*. The residue was dissolved in H_2O (10 mL), extracted with EtOAc (3 x 10 mL), and the aqueous layer was acidified to pH 2 using 6 M HCl. The acidic solution was extracted with EtOAc (3 x 10 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by reverse phase HPLC (C_{18} Bondpak, gradient elution: 0-100% MeCN in H_2O over 20 min) to afford **185** (t_R 18.2 min, 165 mg, 70%) as an oil: $[\alpha]_D^{26}$ +27.0 (*c* 0.1, MeOH); IR (MeOH, cast) 3415, 2952, 1732, 1716, 1695, 1455 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.88-2.40 (m, 4H, C<u>H</u>$_2$C<u>H</u>$_2$), 4.99 (m, 1H, C<u>H</u>NCbz), 5.10 (m, 2H, OC<u>H</u>$_2$Ph), 6.14 (m, 1H, C<u>H</u>=C), 7.26-7.38 (m, 5H, Ph); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 21.4, 27.2, 58.1, 69.1, 123.9,

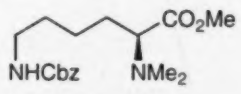
128.7, 128.9, 129.0, 129.4, 133.4, 137.4, 155.9, 170.1, 176.8; HRMS (ES) Calcd for $C_{15}H_{15}NO_6Na$ 328.0797, found 328.0792, $[M+Na]^+$.



Dimethyl (2*RS*,6*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-(2-trimethylsilylethylsulfonyl)amino))-6-(*N*,*N*-dimethylamino)heptane-1,7-dioate (190ab). To a solution of **133ab** (55.0 mg, 0.11 mmol) in MeOH (1 mL) at rt, was added 37% aqueous formaldehyde (55 µL, 0.68 mmol). The solution was stirred for 15 min, treated with sodium cyanoborohydride (11 mg, 0.17 mmol) and stirred for 4 h. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography (30% EtOAc in hexanes) to give a mixture of diastereomers **190a** and **190b** (45 mg, 78%) as a colourless oil: IR ($CHCl_3$, cast) 2951, 1730, 1456, 1435, 1352, 1251, 1139 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 9 H, $Si(C\underline{H}_3)_3$), 0.90-2.20 (m, 17H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2Si$, $C(C\underline{H}_3)_3$), 2.38 (s, 6H, $N(C\underline{H}_3)_2$), 3.18 (m, 1H, $C\underline{H}N(CH_3)_2$), 3.40-3.60 (m, 2H, $C\underline{H}_2SO_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 3.74 (s, 3H, $OC\underline{H}_3$), 4.79 (m, 1H, $C\underline{H}NSO_2$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -2.0, 9.7, 23.1, 27.9, 28.9, 30.3, 41.7, 50.8, 51.2, 52.5, 54.1, 59.0, 67.2, 85.0, 151.0, 170.6; HRMS (ES) Calcd for $C_{21}H_{43}N_2O_8SiS$ 511.2509, found 511.2509, $[MH]^+$.

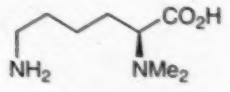


Dimethyl (2*RS*,6*S*)-2-(*N*-(*tert*-butoxycarbonyl)amino)-6-(*N*,*N*-dimethylamino)-heptane-1,7-dioate (191ab). To a solution of **190ab** (30.0 mg, 0.059 mmol) in THF (1 mL) was added 1.0 M solution of TBAF (176 μL, 0.18 mmol) in THF. The resulting solution was stirred for 2 h, and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (60% EtOAc in hexanes) to give a mixture of diastereomers **191a** and **191b** as a colourless oil (18 mg, 90%): IR ($CHCl_3$ cast) 3372, 2951, 1715, 1455, 1366, 1249, 1164 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.30-1.88 (m, 15H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 2.35 (s, 6H, $N(C\underline{H}_3)_2$), 3.18 (m, 1H, $C\underline{H}N(CH_3)_2$), 3.71 (s, 6H, 2 x $OC\underline{H}_3$), 4.09 (m, 1H, $C\underline{H}$NHBoc); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 23.5, 28.7, 30.1, 32.3, 42.0, 51.7, 52.6, 54.8, 68.6, 80.6, 158.1, 173.9, 174.9; HRMS (ES) Calcd for $C_{16}H_{31}N_2O_6$ 347.2182, found 347.2177, $[MH]^+$.



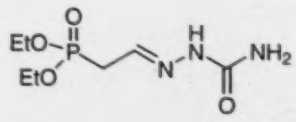
Methyl (2*S*)-2-(*N*,*N*-dimethylamino)-6-(*N*-(benzyloxycarbonyl)amino)hexanoate (193). To a solution of *N*-ε-(benzyloxycarbonyl)amino)-L-lysine hydrochloride (0.50 g, 1.50 mmol) in MeOH (20 mL) at rt was added 37% aqueous formaldehyde (0.73 mL, 9.10 mmol). The solution was stirred for 15 min, and then sodium cyanoborohydride (0.14 g, 2.30 mmol) was added. The solution was stirred for 4 h at rt, and the solvent was

removed *in vacuo*. The residue was purified by flash chromatography (30% EtOAc in hexanes) to give a colourless oil **193** (0.39 g, 80%): $[\alpha]_D^{26}$ -8.5 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$, cast) 3331, 2951, 1719, 1529, 1454, 1249, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.28-1.88 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.45 (s, 6H, $N(C\underline{H}_3)_2$), 3.17 (m, 2H, $C\underline{H}_2NHCbz$), 3.30 (m, 1H, $C\underline{H}N(CH_3)_2$), 3.71 (s, 3H, $OC\underline{H}_3$), 4.82 (br s, 1H, $CHN\underline{H}Cbz$), 5.08 (s, 2H, $OC\underline{H}_2Ph$), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 23.3, 29.0, 29.6, 40.7, 41.3, 51.7, 66.4, 67.0, 128.1, 128.6, 136.7, 156.5; HRMS (ES) Calcd for $C_{17}H_{27}N_2O_4$ 323.1971, found 323.1976, $[MH]^+$.



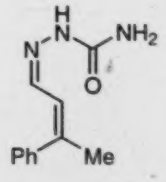
(2*S*)-2-(*N*,*N*-Dimethylamino)-6-aminohexanoic acid (194). A solution of **193** (0.10 g, 0.31 mmol) in MeOH was treated with 10% Pd/C (10 mg) and the mixture was stirred under H_2 at atmospheric pressure for 10 h. The mixture was then filtered through Celite™, and solvent was removed *in vacuo*. The residue was redissolved in MeOH:H_2O (1:1, 5 mL). Lithium hydroxide monohydrate was added (26.0 mg, 0.62 mmol) and the solution was stirred for 6 h. The solvent was removed *in vacuo*, the residue was dissolved in H_2O (5 mL), and the aqueous solution was acidified to pH 2 using 6 M HCl. The solution was concentrated *in vacuo* and the residue was purified by reverse phase HPLC (C_{18} Bondpak, isocratic elution: 2.5% MeCN in H_2O) to give **194** (t_R 2.1 min, 35.0 mg, 65%) as a white solid: $[\alpha]_D^{26}$ +17.1 (*c* 0.1, H_2O); IR (μscope) 3000-2500, 2221, 1593, 1471, 1173 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.39-2.05 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.94 (s,

6H, $N(C\underline{H}_3)_2$), 3.04 (m, 2H,$C\underline{H}_2NH_2$), 3.86 (m, 1H, $C\underline{H}N(CH_3)_2$); ^{13}C NMR (D_2O, 75.5 MHz) δ 22.7, 27.3, 27.4, 39.9, 41.0, 69.6, 172.3; HRMS (ES) Calcd for $C_8H_{19}N_2O_2$ 175.1447, found 175.1450, $[MH]^+$.

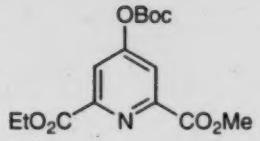


(Diethoxyphosphinyl)acetaldehyde semicarbazone (207).[203] A 2.5 M solution of *n*-butyllithium (3.1 mL, 7.9 mmol) was placed in a dry flask under argon at –78 °C. Dry THF was added (7 mL) and the solution was stirred for 15 min. A solution of diethyl methanephosphonate (1.0 g, 6.6 mmol) in THF (2 mL) was then added dropwise at –78 °C. After 30 min, a solution of DMF (0.61 mL, 7.9 mmol) in THF (5 mL) was added dropwise. The reaction mixture was warmed to rt, and the aqueous and organic layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo* to give a colourless oil. A solution of semicarbazide hydrochloride (0.73 g, 6.6 mmol) in 10 mL of H_2O was added to potassium hydroxide (0.34 g, 6.6 mmol). The colourless oil was added to this solution and the mixture was stirred overnight at rt. The product was extracted with CH_2Cl_2 (3 x 30 mL), and the solvent was evaporated to give a white solid. This was purified by column chromatography (10% MeOH in EtOAc) to afford **207** as a white solid (1.2 g, 80%): IR (μscope) 3378, 3192, 1691, 1605, 1521, 1265, 1019 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.32 (t, 6H, J = 6.0 Hz, 2 x $C\underline{H}_3CH_2O$), 2.92 (m, 2H,

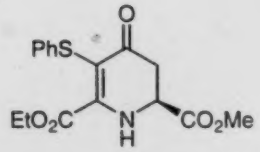
C<u>H</u>$_2$CH=N), 4.15 (q, 4H, J = 6.0 Hz, 2 x CH$_3$C<u>H</u>$_2$O), 7.15 (m, 1H, C<u>H</u>=N); ^{13}C NMR (CD$_3$OD, 75.5 MHz) δ 16.7, 64.1, 136.5, 159.9; HRMS (ES) Calcd for $C_7H_{17}N_3O_4P$ 238.0957, found 238.0956, $[MH]^+$. <u>Note</u>: Methylene protons at 2.92 ppm are exchangable in deuterated solvent after several hours, thus this carbon signal is not observed at 21 ppm in the ^{13}C spectrum.



3-Phenyl-2-butenalsemicarbazone (222). This known compound was synthesized according to a modified procedure.[206] A solution of semicarbazone **207** (47 mg, 0.20 mmol) in dry THF (2 mL) was treated with a 1 M solution of sodium hexamethyldisilazane (210 µL, 0.21 mmol) in THF and stirred for 20 min. The solution was then transferred *via* cannula to a solution of acetophenone (19 µL, 0.17 mmol) in THF (0.5 mL). The solution was allowed to stir overnight and the solvent was removed *in vacuo* to give an oil. This was purified by flash chromatography (80% EtOAc in hexanes) to afford **222** as a white solid (3 mg, 8%): IR (µscope) 3346, 1735, 1684, 1586, 1508, 1255, 1157 cm^{-1}; ^{1}H NMR (CD$_3$OD, 300 MHz) δ 2.25 (s, 3H, C<u>H</u>$_3$), 6.55 (m, 1H, C<u>H</u>=CPh), 7.20-7.55 (m, 5H, Ph), 8.0 (d, 1H, J = 9.0 Hz, C<u>H</u>=N); ^{13}C NMR (CD$_3$OD, 75.5 MHz) δ 16.3, 116.0, 124.4, 126.7, 129.1, 129.5, 142.8, 143.9, 171.2; HRMS (ES) Calcd for $C_{11}H_{13}N_3ONa$ 226.0956, found 226.0956, $[M+Na]^+$.

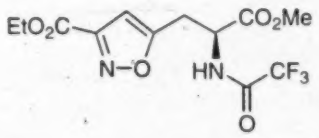


2-Carboethoxy-6-carbomethoxy-6-methyl 4-(*tert*-butoxycarbonyloxy)pyridine (223). To a solution of **82** (35 mg, 0.14 mmol) and di-*tert*-butyl pyrocarbonate (34 mg, 0.16 mmol) in dry THF (2 mL) was added dropwise a 1 M solution of sodium hexamethyldisilazane (157 µL, 0.16 mmol) in THF. The resultant dark red solution was stirred overnight. The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography (25% EtOAc in hexanes) to give **223** as a solid (19 mg, 40%): IR (µscope) 3098, 1765, 1718, 1596, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.43 (t, 3H, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$), 1.57 (s, 9H, $C(C\underline{H}_3)_3$), 4.00 (s, 3H, $OC\underline{H}_3$), 4.47 (q, 2H, *J* = 7.2 Hz, $OC\underline{H}_2CH_3$), 8.13 (d, 1H, *J* = 2.1 Hz, C=C<u>H</u>), 8.15 (d, 1H, *J* = 2.1 Hz, C=C<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.3, 27.7, 53.3, 62.7, 85.7, 120.5, 120.6, 149.62, 150.3, 150.6, 159.8, 163.9, 164.6; HRMS (ES) Calcd for $C_{15}H_{19}NO_7Na$ 348.1059, found 348.1058, $[M+Na]^+$.



(6*S*)-2-Carboethoxy-6-carbomethoxy-4-oxo-5-phenylsulfenyl-1,2,3,4-tetrahydro pyridine (227). To solution of **82** (25 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) was added

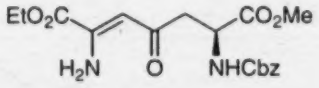
propylene oxide (11 μL, 0.16 mmol) and crushed 3 Å molecular sieves. The mixture was stirred for 15 min at 0 °C, and then phenylsulfenyl chloride (25 μL, 0.21 mmol) was added. The solution became immediately bright yellow in colour. The reaction mixture was stirred for an additional 1 h at rt, and the solvent was removed *in vacuo*. Purification of the residue by column chromatography (60% EtOAc in hexanes) afforded **227** as a yellow foam (20 mg, 55%): $[\alpha]_D^{26}$ +69.6 (*c* 0.5, CH_2Cl_2); IR (μscope) 3226, 3057, 1742, 1641, 1596, 1581, 742, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.18 (t, 3H, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$), 2.84 (dd, 1H, *J* = 16.5, 13.2 Hz, $C\underline{H}_aH_bCHNH$), 2.98 (ddd, 1H, *J* = 16.5, 5.4, 1.5 Hz, $CH_a\underline{H}_bCHNH$), 3.83 (s, 3H, $OC\underline{H}_3$), 4.28 (q, 2H, *J* = 7.2 Hz, $OC\underline{H}_2CH_3$), 4.49 (ddd, 1H, *J* = 13.2, 5.4, 1.5 Hz, $C\underline{H}NH$), 6.25 (s, 1H, $N\underline{H}$), 7.05-7.20 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.4, 39.2, 54.1, 55.1, 63.9, 101.4, 126.1, 127.4, 129.3, 138.4, 157.7, 163.7, 170.4, 188.5; HRMS (ES) Calcd for $C_{16}H_{17}NO_5NaS$ 358.0725, found 358.0729, $[M+Na]^+$.



Methyl (2*S*)-2-(*N*-(2,2,2-trifluoroacetyl)amino)-3-(3-(ethoxycarbonyl)-2-isoxazole) propanoate (228). A solution of isoxazole **80** (0.75 g; 2.20 mmol) in CH_2Cl_2 (20 mL) was treated with TFA (2 mL, 22.0 mmol) dropwise and stirred for 1.5 h. The solvent was evaporated and the resultant oil was dried *in vacuo* overnight. To this residue was added CH_2Cl_2 (5 mL), triethylamine (620 μL, 4.40 mmol) and benzyl chloroformate (376 μL,

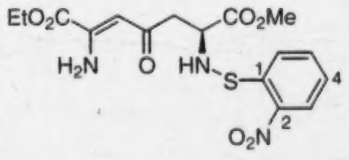
2.6 mmol). The resulting solution was stirred at rt for 3 h, and then added to 1 M HCl (50 mL). The mixture was extracted with CH_2Cl_2 (4 x 35 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a yellow oil which was purified by HPLC (C_{18} Bondpak reverse phase, 8 x 200 mm, gradient elution: 0-80% MeCN in H_2O containing 0.1% TFA) to give **228** as a colourless oil (t_R 11.6 min, 150 mg, 20%) and **51** (t_R 14.1 min, 620 mg, 75%).

For **228**: $[\alpha]_D^{26}$ +85.0 (*c* 0.3, CH_2Cl_2); IR (CH_2Cl_2, cast) 3323, 1729, 1598, 1552 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.37 (t, 3H, *J* = 8.0 Hz, $C\underline{H}_3CH_2O$), 3.42 (dd, 1H, *J* = 15.6, 5.1 Hz, $C\underline{H}_aH_bCHNH$), 3.55 (dd, 1H, *J* = 15.3, 5.1 Hz, $CH_a\underline{H}_bCHNH$), 3.75 (s, 3H, $OC\underline{H}_3$), 4.40 (q, 2H, *J* = 8.0 Hz, $CH_3C\underline{H}_2O$), 4.88 (m, 1H, $C\underline{H}NH$)), 6.47 (s, 1H, $C\underline{H}$=C), 7.10 (s, 1H, $N\underline{H}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.1, 28.5, 51.2, 53.6, 62.4, 104.1, 117.0, 156.7, 157.3, 159.6, 168.7, 169.1; HRMS (ES) Calcd for $C_{12}H_{13}N_2O_6F_3Na$ 361.0623, found 361.0622, $[M+Na]^+$. All spectroscopic data obtained for **51** using this procedure was consistent with previously reported data.



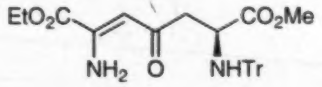
1-Ethyl 7-methyl (6*S*)-2-amino-6-(*N*-(benzyloxycarbonyl)amino)-4-oxo-2-heptene-1,7-dioate (229). A procedure for reductive ring opening of isoxazoles was adapted.[141] To a solution of isoxazole **51** (146 mg, 0.39 mmol) in MeCN (5 mL) was added molybdenum hexacarbonyl (67 mg, 0.25 mmol) and H_2O (5 μL, 0.47 mmol). The

reaction mixture was heated to reflux under argon for 5 h, during which time the solution became dark brown. The solvent was evaporated *in vacuo* to afford an oil which was purified by flash chromatography (10-35% EtOAc in hexanes) to give **229** as a light yellow oil (95 mg, 65%): $[\alpha]_D^{26}$ +73.0 (*c* 0.4, CH_2Cl_2); IR (CH_2Cl_2, cast) 3425, 3301, 1726, 1637, 1591, 1271 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.34 (t, 3H, *J* = 7.2 Hz, $C\underline{H}_3CH_2O$), 2.97 (dd, 1H, *J* = 17.4, 4.5 Hz, $C\underline{H}_aH_bCHNH$), 3.20 (dd, 1H, *J* = 17.4, 4.8 Hz, $CH_a\underline{H}_bCHNH$), 3.72 (s, 3H, $OC\underline{H}_3$), 4.30 (q, 2H, *J* = 7.2 Hz, $CH_3C\underline{H}_2O$), 4.58 (m, 1H, $C\underline{H}NH$)), 5.10 (s, 2H, $OC\underline{H}_2Ph$), 5.84 (s, 1H, $C\underline{H}$=C), 7.26-7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.9, 43.9, 50.4, 52.5, 62.7, 66.9, 95.6, 127.9, 128.0, 128.4, 136.3, 146.4, 156.1, 163.5, 172.1, 197.8; HRMS (ES) Calcd for $C_{18}H_{22}N_2O_7Na$ 401.1325, found 401.1327, $[M+Na]^+$.



1-Ethyl 7-methyl (6*S*)-2-amino-4-oxo-6-(*N*-(2-nitrophenylsulfenyl)amino)-2-heptene-1,7-dioate (231). A solution of vinylogous amide **83** (31 mg, 0.13 mmol) in THF (2 mL) was treated with triethylamine (35 μL, 0.25 mmol) followed by the addition of *o*-nitrophenylsulfenyl chloride (26 mg, 0.14 mmol). The resultant solution was stirred overnight at rt, and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (50% EtOAc in hexanes) to give the product as a yellow oil (20 mg, 40%): $[\alpha]_D^{26}$ +44.7 (*c* 0.5, CH_2Cl_2); IR ($CHCl_3$, cast) 3312, 1732, 1633, 1591 cm^{-1}; ^1H

NMR ($CDCl_3$, 600 MHz) δ 1.34 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 3.02 (dd, 1H, J = 8.4, 3.0 Hz, $C\underline{H}_aH_bCHNH$), 3.06 (dd, 1H, J = 8.4, 2.7 Hz, $CH_a\underline{H}_bCHNH$), 3.78 (s, 3H, $OC\underline{H}_3$), 3.88 (m, 1H, $C\underline{H}NH$)), 4.32 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 5.82 (s, 1H, $C\underline{H}$=C), 7.20 (m, 1H, 4-$C\underline{H}$ Ph), 7.54 (m, 1H, 6-$C\underline{H}$ Ph), 8.02 (m, 1H, 5-$C\underline{H}$ Ph), 8.22 (m, 1H, 3-$C\underline{H}$ Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1, 44.9, 52.7, 60.5, 62.8, 97.1, 124.7, 124.9, 125.7, 133.8, 142.7, 145.2, 146.5, 163.6, 173.5, 197.6; HRMS (ES) Calcd for $C_{16}H_{19}N_3O_7NaS$ 420.0841, found 420.0836, $[M+Na]^+$.



1-Ethyl 7-methyl (6*S*)-2-amino-4-oxo-6-(*N*-(trityl)amino)-2-heptene-1,7-dioate (233). A solution of vinylogous amide **83** (50 mg, 0.21 mmol) in THF (2 mL) was treated with triethylamine (43 μL, 0.31 mmol) followed by trityl chloride (63 mg, 0.23 mmol). The resultant solution was stirred overnight, and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (50% EtOAc in hexanes) to furnish **233** as an oil (47 mg, 57%): $[\alpha]_D^{26}$ +50.0 (*c* 0.1, CH_2Cl_2); IR ($CHCl_3$, cast) 3440, 3055, 1732, 1695, 1685, 1651, 1592, 1207 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (t, 3H, J = 9.0 Hz, $C\underline{H}_3CH_2O$), 2.57 (dd, 1H, J = 15.0, 9.0 Hz, $C\underline{H}_aH_bCHNH$), 2.70 (dd, 1H, J = 15.0, 6.0 Hz, $CH_a\underline{H}_bCHNH$), 3.22 (s, 3H, $OC\underline{H}_3$), 3.70 (m, 1H, $C\underline{H}NH$)), 4.32 (q, 2H, J = 9.0 Hz, $CH_3C\underline{H}_2O$), 5.90 (s, 1H, $C\underline{H}$=C), 7.10-7.50 (m, 15H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ

14.1, 48.4, 51.8, 54.4, 62.7, 71.3, 97.1, 126.5, 127.9, 128.9, 145.9, 146.0, 163.9, 174.7, 198.6; HRMS (ES) Calcd for $C_{29}H_{30}N_2O_5Na$ 509.2052, found 509.2049, $[M+Na]^+$.

3. Enzyme Purification and Assays

Inhibition Studies with DAP D-Dehydrogenase

DAP D-dehydrogenase was isolated and purified from *Bacillus spaericus* IFO 3525 as previously reported.[50,52,57] Purity of the enzyme samples was greater than 90% as determined by SDS-Page analysis (data not shown). Spectrophotometric assays were performed on a GBC Cintra 40 UV spectrophotometer equipped with a Neslab RTE-111 variable temperature bath. The enzyme was assayed at 25.0 °C in 0.1 M Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 1.10 mM *meso*-DAP and 0.3 mM NADP at pH 7.8 in a total volume of 1 mL. Pure DAP isomers (LL and *meso*) were obtained by enzymatic resolution by Dr. Yong Gao, as previously described.[111]

The increase of absorbance at 340 nm, using a 1 cm light path was then followed. One unit of enzyme activity is defined as the production of 1 μmol of NADPH produced per min under the assay conditions. For examination of the inhibitors as substrates, each assay mixture contained a constant inhibitor concentration of 2.5 mM and 1.8 mU of enzyme, with omission of *meso*-DAP. For inhibition tests, the assay buffer contained varying concentrations of inhibitor (100 μM-1 mM), 1.06 mM *meso*-DAP, and 3.6 mU of enzyme. These results were compared against a control performed simultaneously, where the Tris-HCl buffer replaced the inhibitor.

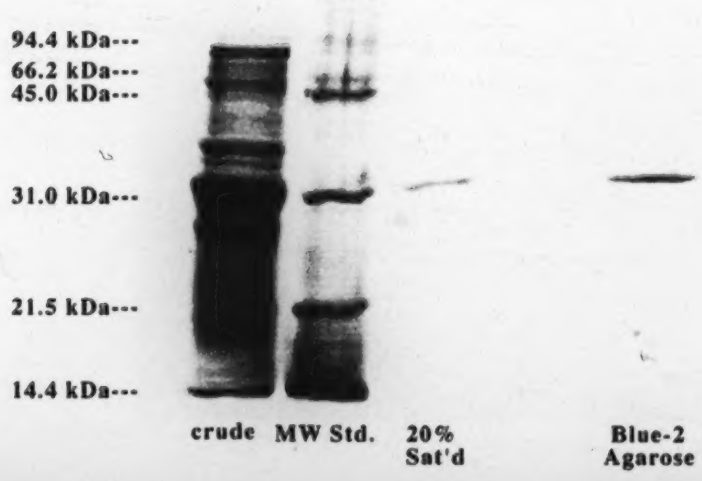
Inhibition Studies with DAP Epimerase

DAP epimerase was isolated and purified from an *E. coli* BL21 (DE3) pLysS mutant (Strain engineered by Bruce Malcolm, Schering Plough) by modification of the literature procedure.[58] Cultures were grown on solid agar media containing ampicillin (0.1 mg/mL) at 37 °C for 13 h. Individual colonies were transferred from the plate to a 1 L shake flask and the culture was allowed to grow for 14.5 h at 37 °C. Subsequently, this *E. coli* culture was used to inoculate a fermenter containing Luria Broth (10 L) and ampicillin (0.1 mg/mL). The growth of the culture was monitored spectrophotometrically at 600 nm until the optical density reached 0.6 absorbance units. At this time, the culture was induced with isopropyl-β-D-thiogalactoside (IPTG) (0.5 mM final concentration) and incubated for 6 h at 37 °C. The bacteria were harvested, and the pellet was washed with potassium phosphate buffer (200 mL, 30 mM). The cells were resuspended in 20 mM potassium phosphate (pH 7.2) containing 1 mM EDTA and 10 mM DTT. The paste was then divided into five aliquots of 150 mL. All subsequent operations were performed at 4 °C. Approximately 750 mL of this bacterial solution was lysed using a French Pressure Cell (AMINCO) at 20,000 psi.

The crude lysate was centrifuged at 5000 rpm and the supernatant was decanted. The pellet was rinsed with buffer and the resultant solution (800 mL) was concentrated to 150 mL using an Amicon Diaflo membrane system. The concentrate was dialyzed for 15 h with 5 mM potassium phosphate buffer (1 mM EDTA, 10 mM DTT, pH 7.2). The crude, dialyzed enzyme solution was further applied to a DEAE-52 cellulose column (4 x 25 cm) at 1.0 ml/min, flushed with buffer A (200 mL), and then the epimerase activity

was eluted using a continuous linear gradient (4 L) from 5 mM to 100 mM potassium phosphate (1 mM EDTA, 10 mM DTT, pH 7.2) at a flow rate of 3.0 ml/min. Protein concentration was followed by absorbance at 280 nm. All fractions containing active enzyme were combined and concentrated to a final volume of 60 mL. Thereafter, the enzyme solution was brought to 20% saturation with $(NH_4)_2SO_4$. The solution was allowed to stir for 1 h and then centrifuged at 20,000 rpm for 65 min. The pellet obtained was separated from the supernatant and dissolved in 30 mL of 100 mM phosphate buffer (1 mM EDTA, 10 mM DTT, pH 8.0). This solution was then dialyzed against the same buffer three times over 18 h (2.5 L dialyzing buffer in total). The dialyzed solution was then applied to a Reactive Blue-2 agarose column (4 ml bed volume) equilibrated in the same buffer, and the activity was eluted with the same buffer containing 0.5 M KCl. All active fractions were combined, concentrated to 40 mL and dialyzed against 100 mM phosphate buffer (1 mM EDTA, 10 mM DTT, pH 7.2). The final specific activity was 75 U/mg (See below). A 12% Tris-HCl SDS PAGE gel electrophoresis is shown in Figure 20.

Figure 20. Coomassie blue-stained 12% Tris-HCl SDS PAGE gel electrophoresis of DAP epimerase.



Epimerase activity was monitored using a coupled enzyme assay, which monitors the production of NADPH at 340 nm at 25 °C. The assay is performed in a 1 mL quartz cuvette filled with 1 mL of buffer solution (0.1 M Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.8) containing 0.4 mM LL-DAP, 0.3 mM NADP, and 0.06 units of DAP D-dehydrogenase. One unit (U) of epimerase activity corresponds to the production of one µmol of NADPH per minute. For inhibition studies with inhibitors, the assay buffer contained varying concentrations of inhibitor (0.100 mM-1 mM), 0.4 mM LL-DAP, 0.3 mM NADP, 18-50 mU of DAP dehydrogenase, and 15 mU of DAP epimerase. These results were compared against a control performed simultaneously, where the Tris-HCl buffer replaced the inhibitor.

Test of Analogues as Substrates for DAP Epimerase

Compounds were tested as substrates for epimerase by incubating 700 mU of active and inactive epimerase in the presence of inhibitor (3.0 mM, 9 µmol) in deuterated buffer (0.1 M Tris-DCl, 1 mM EDTA, 1 mM DTT, pD 7.8) in a final volume of 3.0 mL at 25 °C for 18 h. Freezing in dry ice/isopropanol bath quenched the incubations. The mixtures were then thawed and loaded onto ion exchange columns (1.0 mL, BioRad AG1X8, OH^- form, 100-200 mesh). The columns were rinsed with water (5 mL), and the substrate analogue was eluted with 1 M HCl. The solvent was evaporated *in vacuo*, and the residue was dissolved in D_2O and proton NMR spectra were acquired. Integration of the α-proton signals indicated whether substitution of deuterium had taken place. The

samples were then lyophilized, the residues were dissolved in water, and the water was subsequently removed *in vacuo*. This step was repeated twice, the residues were redissolved in H_2O and the ^2H NMR spectra were obtained using a Bruker WH-400 MHz instrument.

Purification of *meso*-DAP Decarboxylase

DAP decarboxylase was isolated and purified from *Bacillus spaericus* IFO 3525 by modification of the literature procedure.[116] *B. spaericus* IFO 3525 was grown in 20 L cultures as described previously.[117] The purification of the enzyme was performed at 4 °C and potassium phosphate buffer containing 0.1 mM pyridoxal phosphate, 0.01% 2-mercaptoethanol, and 1 mM EDTA was used throughout, unless otherwise specified. The washed cells (80 g wet weight) were suspended in 800 mL of 20 mM buffer and disrupted using a French Pressure Cell (AMINCO) at 20,000 psi. The supernatant solution obtained by centrifugation (8000 rpm for 60 min) was fractionated with ammonium sulfate (40~60% saturation). The pellet obtained from 60% saturation was suspended in 20 mM buffer (200 mL). The enzyme solution was dialyzed overnight against 100 volumes of 10 mM buffer. To the dialyzed enzyme solution was added 1.0 mL of 2% aqueous protamine sulfate solution per 100 mg of protein (5 ml total was added). After 20 min of stirring, the precipitate was removed by centrifugation (10 g, 30 min). The supernatant solution was then dialyzed against 100 volumes of 10 mM acetic acid-sodium acetate buffer (pH 5.0) containing 0.1 mM PLP, 0.01% 2-mercaptoethanol, and 1 mM EDTA. The insoluble materials that formed during dialysis were removed by

centrifugation. The enzyme solution was adjusted to pH 6.8 with 28% ammonia and applied, at a flow rate of 0.8 ml/min, to a DEAE-cellulose column (2.5 cm x 16 cm) equilibrated with 10 mM buffer containing 20% glycerol. After the column was washed with the same buffer, followed by buffer containing 0.1 M NaCl, the enzyme was eluted with the buffer containing 0.15 M NaCl. The active fractions were combined (400 mL total) and dialyzed overnight against 10 mM buffer containing 0.1 mM PLP and 0.01% 2-mercaptoethanol to remove glycerol and EDTA. The enzyme solution was then concentrated to a final volume of 10 mL by ultrafiltration using an Amicon 2000. The enzyme was then applied to a hydroxyapatite column (2.5 cm x 13 cm) equilibrated with the above dialysis buffer. The column was washed with the same buffer, then with 20 mM buffer and finally the enzyme eluted with 30 mM buffer (pH 6.8). The active fractions were concentrated by ultrafiltration to a final volume of 10 mL and glycerol was added to a final concentration of 20%. The enzyme solution was then applied to a Sephadex G-150 packed column (1.5 cm x 50 cm) equilibrated with 10 mM buffer containing 20% glycerol and eluted with the same buffer. The active fractions were pooled and concentrated. At this point a second Sephadex G-150 column was performed in the same manner. Approximately 500–fold purification was achieved at this stage with an overall yield of 2%. A summary of the purification is presented in Table 3. The purified protein showed three protein bands on a 12% Tris-SDS PAGE electrophoresis gel (Figure 21). All substrate and inhibition tests were performed with the enzyme at this level of purity.

Figure 21. Coomassie blue-stained 12% Tris-SDS PAGE electrophoresis gel of DAP decarboxylase.

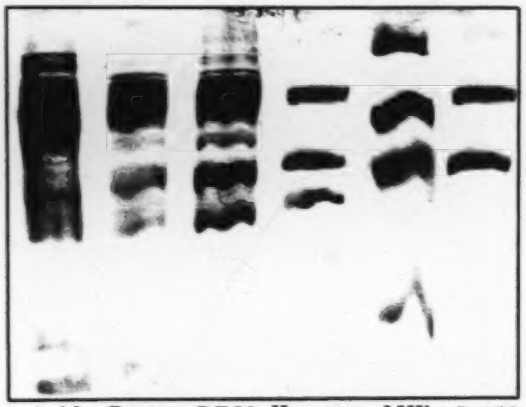
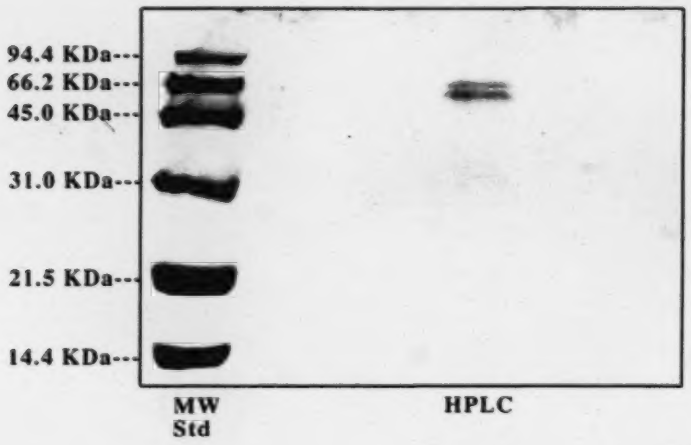


Table 3. Purification of DAP Decarboxylase.

Step	Protein (mg/mL)	Specific Activity(U/mg)	Total Units	Yield (%)
Crude Extract	8.2	0.02	105	100
60% sat'd	4.8	0.03	51	48
Protamine	2.5	0.08	38	36
Acid	0.9	0.16	36	34
DE52	6.3	0.12	7	7
Hydroxyapatite	0.3	1.1	5	4.8
G-150 sephadex	0.1	2.0	4	3.8
G-150 sephadex	0.02	13.0	2	2

A further attempt to purify the enzyme to homogeneity was accomplished using reverse phase HPLC (C_8, 300 Å, gradient elution, 5-50% buffer (5 mM phosphate buffer, 0.01% 2-mercaptoethanol in MeCN over 20 min, t_R 12.5 min). Gel electrophoresis revealed one major band having a molecular weight of 59 kDa as determined by molecular weight markers (Figure 22).

Figure 22. Coomassie blue-stained 12% Tris-SDS PAGE electrophoresis gel of DAP decarboxylase purified by HPLC.



Inhibition Studies with *meso*-DAP Decarboxylase

The enzyme activity was assayed by the end-point determination of L-lysine using the commercially available enzyme saccharopine dehydrogenase (Sigma).[118] All inhibitors were first tested as inhibitors of saccharopine dehydrogenase using a continuous spectrophotometric assay which follows a decrease in absorbance of NADH

at 340 nm. The control assay mixture consisted of 100 mM phosphate buffer (1 mM EDTA, 0.01% 2-mercaptoethanol, pH 6.8), 0.3 µmol of NADH, 2.5 µmol of α-ketoglutarate, and 25 mU of saccharopine dehydrogenase in a final volume of 300 µL. Inhibition tests consisted of the above assay mixture containing 0.5-1.0 mM DAP analogue.

For determination of *meso*-DAP decarboxylase activity, the assay mixture contained in a total volume of 300 µL: 100 mM phosphate (1 mM ETDA, 0.01% 2-mercaptoethanol, pH 6.8), 0.1 mM PLP, 1.2 mM *meso*-DAP and enzyme. For the blank test, the *meso*-DAP was replaced by water. The assay mixture was incubated for 30 min at 37 °C, then boiled for 1 min in a water bath to terminate the reaction. This was then cooled to room temperature, 0.3 µmol of NADH, 2.5 µmol of α-ketoglutarate, 150 mU of saccharopine dehydrogenase and 100 mM buffer were added to a final volume of 1 mL. After 1 h at rt, the absorbance at 340 nm was determined. The amount of NADH consumed was calculated from the difference in absorbance between blank and sample. The amount of lysine formed during the initial 30 min incubation in the decarboxylation reaction is equivalent to the amount of NADH oxidized by the saccharopine dehydrogenase. Therefore, one unit of *meso*-DAP decarboxylase activity is defined as one µmol of NADH oxidized per min. Inhibition studies with *meso*-DAP decarboxylase involved spectrophotometric determination of NADH oxidation using the above discontinuous assay mixture containing 0.35-2.0 mM inhibitor.

Assay for Time-Dependent Inhibition of *meso*-DAP Decarboxylase with Substrate Analogues

For analogues which showed inhibition of DAP decarboxylase, time dependence was investigated. The analogue (3 mM) was incubated with enzyme (75 mU) at 25 °C. Aliquots (40 µL) were removed from this mixture at 0, 30, 60, 90 and 120 min, and were added to an assay mixture (300 µL) containing 100 mM phosphate (1 mM ETDA, 0.01% 2-mercaptoethanol, pH 6.8), 0.1 mM PLP, and 1.2 mM *meso*-DAP. The assay mixture was incubated at 37 °C, boiled for 1 min and amount of L-lysine produced was determined as described previously using saccharopine dehydrogenase.

Radioactive Assay of *meso*-DAP Decarboxylase

Diaminopimelate decarboxylase activity was estimated by measuring the $^{14}CO_2$ evolution from [1,7-^{14}C]-DAP as described by Kelland *et al.*[89] Radioactivity was determined using standard liquid scintillation procedures in 10 mL plastic scintillation vials (Terochem) with Beckman Ready gel scintillation cocktail (Fullerton, CA). A Beckman LS 5000TD instrument equipped with automatic quench control was used to directly determine decompositions per min (dpm) in radioactive samples, by comparison against quench curves prepared from Beckman ^{14}C quenched standards. The assay mixtures (in scintillation vials) contained 100 mM phosphate buffer, pH 6.8, 1.2 mM *meso*-DAP, 50 µM PLP, 1.2 mM EDTA, 0.01% 2-mercaptoethanol, 0.25 µCi of [1,7-^{14}C]

diaminopimelate (American Radiolabelled Chemicals Inc.) and enzyme in a final volume of 0.86 mL. The caps of the vials contained a 1.5 cm x 1.5 cm pieces of filter paper impregnated with 30 µL of 1 M hyamine hydroxide as a $^{14}CO_2$ trapping agent. The reaction was initiated by the addition of enzyme and the assay mixture was incubated at 30 °C with continual shaking for 20 min. Reaction was terminated by the addition of 0.2 mL of 10% trichloroacetic acid and the vials were shaken for an additional 60 min to ensure the evolution of $^{14}CO_2$ was complete. The filter papers were removed and counted in 10 mL of ACS scintillation cocktail. One unit of enzyme activity is defined as the amount of enzyme that liberates 1 µmol of CO_2 per minute. Inhibition tests were performed in an analogous manner with inclusion of 1.0 mM substrate analogue.

Synthesis of L-Aspartate Semialdehyde (for DHDP Reductase Studies)

These experiments were performed by Renjian Zheng, and Prof. John Blanchard, Dept. of Biochemistry, Albert Einstein College of Medicine. L-Aspartate semialdehyde (L-ASA) was prepared by the ozonolysis of L-allyl-glycine according to the method of Black and Wright in 1 N HCl at 0 °C.[213] L-ASA was purified by applying the solution to a 1 cm x 30 cm AG-X8 cation-exchange column (H^+ form), washing with water and eluting with a 0-1 M HCl gradient. Fractions containing L-ASA were pooled and concentrated *in vacuo*, and the final concentration of L-ASA was determined enzymatically using aspartate semialdehyde dehydrogenase.

Inhibition Studies with DHDP Reductase

These experiments were performed by Renjian Zheng, and Prof. John Blanchard, Dept. of Biochemistry, Albert Einstein College of Medicine. Determination of the initial rate of DHDP reductase activity was based on following the decrease in absorbance at 340 nm of NADPH using 1 cm pathlength quartz cuvettes in a Gilford 260 spectrophotometer maintained at 25 °C with a circulating water bath and thermospacers. Typical assays contained 100 mM Hepes, pH 7.8, 100 μM NADPH, 1 mM pyruvic acid, 10 μg of dihydrodipicolinate synthase, 25-100 μM L-ASA and 0-200 μM concentrations of inhibitor. Reaction mixtures were incubated for five minutes to allow for the complete conversion of pyruvate and L-ASA to dihydrodipicolinate and reactions initiated by the addition of a small volume (2-10 μL) of dihydrodipicolinate reductase.

Data Analysis for DHDP Reductase

Reciprocal initial velocities were plotted against the reciprocal concentration of L-DHDP at various inhibitor concentrations and the data fitted to the equation describing competitive inhibition: $1/v = K_m(1 + I/K_i)/ (V_{max}* [DHP]) + 1/V_{max}$ where v is the initial velocity, [DHP] is the concentration of L-DHDP, I is the concentration of inhibitor, K_i is the slope inhibition constant and V_{max} is the maximal velocity.

Inhibition Studies with DAP Desuccinylase

The cloning, overexpression, and purification of the *dapE*-encoded desuccinylase from *H. influenzae* was done by the research group of Prof. J. S. Blanchard as previously described.[75] The substrate used for the assays was a mixture of D,D- and L,L-succinyl DAP. Enzyme activity was monitored by following a decrease in amide bond absorbance due to succinyl DAP desuccinylation at 220 nm in a Hewlett Packard 8452A diode array spectrophotometer equipped with a constant temperature circulating water bath. Assays were performed in 50 mM K_2HPO_4 (pH 7.6) containing 100 μM $ZnSO_4$ at a temperature of 25 °C. Assays were initiated by the addition of enzyme. DAP analogues were initially tested as substrates using 1 mM substrate in place of succinyl-DAP. Compounds were then tested as inhibitors using 2 mM SDAP, 1 mM DAP analogue, enzyme and assay buffer in a total volume of 300 μL.

Inhibition Studies with *meso*-Diaminopimelate-Adding Enzyme

The MurE activity was partially purified from *E. coli* JM83 (pHE5) and DAP analogues tested by the research group of Dr. J. Van Heijenoort[174](Biochimie Moléculaire et Cellulaire, Université de Paris-Sud, France). DAP analogues were tested as either substrates or inhibitors of the enzyme as previously described[176] except that 0.4 mM UDP-MurNAc-L-Ala-D-Glu substrate concentration was used. Percentages of inhibition were determined for each analogue based on a 5 mM concentration.

Antibacterial Assays

The organisms used for the assay included *E. coli*, *B.sphaericus* IFO 3525, *P. vulgaris* X-19-0, and *C. glutamicum* A5019 PR56. Precultures of the organisms were grown in LB media at 37 °C for 5-8 h. A 2% inoculum of each organism in soft agar was then used to prepare LB-agar plates. Solutions (10 µL) of DAP analogues (1mM and 10 mM) were applied to the soft agar plates and the plates incubated at 37 °C for 18 h. Tetracycline (1 mM and 10 mM) and ampicillin (1mM and 10 mM) were also used as antibacterial controls. Inhibition of bacterial growth was measured as a halo around the spot containing the test compound.

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